The American Journal of Human Genetics

Vol. 7 No. 2 *June 1955*

Chromatographic Studies on Urinary Excretion Patterns in Monozygotic and Dizygotic Twins

I. Methods and Analysis

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INTRODUCTION

EXCEPTING MONOZYGOTIC TWINS, no two individuals of man or of any other sexually reproducing and cross-fertilizing species are likely to carry the same gene complements. This genetic diversity is reflected on the morphological level in the pervasive variability of external appearance. Anthropologists and geneticists have for the most part confined their studies to morphological variation. Yet, the morphological variation is only the outward sign of the underlying physiological variation. Physiological differences bring about the individually distinct developmental patterns, which usually, though not necessarily, give rise to morphological differences. Understanding of the physiological variation is, therefore, probably fundamental for an eventual comprehension of the nature and origin of the differences among human beings.

Evidence is accumulating that metabolic differences among human individuals are as ubiquitous as the more easily perceptible morphological differences. Individual differences in the excretory patterns are readily detected by means of suitable methods, and they may prove to be a clue to the more recondite metabolic processes taking place in the body (Williams and collaborators 1951, Harris 1953 b, Berry 1953). Relatively simple techniques for investigation of the urinary excretion patterns have recently been developed, owing to the improvement of the methods of chromatographic analysis on paper of substances contained in urine (Dent 1947, 1948, Williams and collaborators 1951, and others). Although these methods are still admittedly unprecise, they furnish an opportunity for at least a preliminary orientation in the novel field of studies on metabolic variation in man. It should be kept in mind that, at the present state of our knowledge, geneticists and anthropologists have requirements which do not always coincide with those of their physiological and biochemical colleagues. To a geneticist, an opportunity to make a rapid survey of a relatively large number of individuals may prove more valuable than a very precise description of the excretion patterns of a few selected individuals.

The rapidly growing literature on variations in urinary excretion patterns in man has been concerned primarily with two quite distinct lines of investigation. On the one hand it has been concerned with the aberrant metabolic situations found in con-

Received September 14, 1954.

* U.S. Public Health Service Post-Doctoral Fellow.

† Research Aided by Office of Naval Research.

nection with certain diseases (see Harris 1953b, and Ceppellini 1953 for reviews). Both genetically caused and environmentally induced aberrations are known. In fact, the "inborn errors of metabolism" are the classic traits studied by biochemical geneticists in man.

The other main line of investigation has been the variation in excretory patterns of normal individuals (Woodson et al., 1948; Dunn et al., 1947; Berry, 1953; to mention but a few). From these studies it can definitely be concluded that considerable variability exists among the excretion rates of different individuals for most of the substances investigated. In particular Berry (1953), who estimated the concentrations of 23 substances in urine samples of 237 individuals in Texas, found that the variance of a given substance in different samples from the same individual was always smaller, and for some substances much smaller, than the variance in samples from different individuals.

A limited amount of work has been carried out on the effect of diet on urinary excretion patterns, particularly on the effect of varying protein intake on amino acid excretion (Dunn et al., 1949; Eckhardt et al., 1948; Kirsner et al. 1949). The results of these studies are far from conclusive. However, the general impression, in regard to amino acid excretion, has been that the investigated differences in protein intake (usually extreme) cannot account for all the observed variation in amino acid excretion among the individuals studied.

The two different lines of investigation discussed above come together in the study of the effect of heredity on the patterns of excretion in normal individuals. Harris (1953 a) and Calchi-Novati et al., (1953) found that between 5% and 10% of the people whom they examined in England and in Italy respectively excreted considerably greater quantities of beta-amino-isobutyric acid than the rest; the data suggest that the excretors of relatively large amounts of this substance are homozygous for a recessive allele of a gene which has no other known effects. Sutton and Vandenberg (1953) compared the concentrations of 23 substances in urine samples of 16 sibling pairs; the individuals concerned were confined in an institution for mental defectives, and presumably had similar diets. For 12 of the substances the sibs were significantly more similar than were unrelated individuals.

Disregarding the pathological aberrants, most of the substances detected on paper chromatograms are present in the urine of all or of most persons examined, albeit sometimes in very different concentrations. Some substances seem to be lacking in the urine of certain persons, but such apparently qualitative differences may mean only that the methods used are not sensitive enough to detect concentrations below a certain threshold. In any case, the differences between the urinary excretion patterns of most persons are chiefly quantitative. In dealing with quantitative, and at the same time greatly variable, traits, the first problem which confronts a geneticist is that of heritability. We have chosen to approach this problem by comparing the concentrations of a series of substances in the urine samples of monozygotic and of dizygotic twin pairs. Despite all the limitations of the twin method, one should be able to distinguish those substances, the heritability of which will make them promising for further work, from substances less promising for genetic study.

The results are reported in two articles. The individuals examined, the methods of

investigation, and the statistical treatment of the data are described in the present article, using the observations for four amino acids which are detected in considerable concentrations in the urine samples of all the twins examined. Data on other substances are given in the following article.

THE TWINS

Twin subjects for the study of urinary amino acid excretion were obtained, with one exception, through the Twin Project conducted by the Institute for the Study of Human Variation in cooperation with the Constitution Laboratory in the Department of Medicine, Columbia University. This project is for the study of hereditary and environmental factors in body build and has been in progress for approximately two years with support for part of that time from the Commonwealth Fund. In the course of this study all subjects are given thorough physical examinations and data relevant to the determination of zygosity are obtained.

For the purposes of the present study, diagnosis of zygosity of like sex twins was made primarily on the basis of blood grouping, using all available testing sera for the ABO, Rh, Kell, Duffy, Lewis, Kidd, MNS, and P systems. If there was disagreement

TABLE 1.—DESCRIPTION OF TWIN PAIRS WHO FURNISHED URINE SAMPLES

M—monozygotic; D—dizygotic; ?—doubtful; T—living together; S—living separately

Pair No.	Zygosity	Sex	Age	Residence	No. Urine Samples
1	D	♀ ♀	44	S	3
3	D	♂ ♂	22	S	3
5	D	♀ ♀	36	S	3
6	D	♀ ♀	33	S	1
12	D	♀ ♂	22	T	4
14	D	♂ ♂	18	T	1
17	D	♂ ♂	26	T	3
27	D	♀ ♀	18	T	3
29	D	♀ ♀	29	S	3
37	D	♀ ♀	46	S	3
40	D	♀ ♀	35	T	3
2	M	♂ ♂	54	S	3
4	M	♂ ♂	18	T	3
7	M	♀ ♀	20	T	1
8	M	♀ ♀	26	T	3
9	M	♂ ♂	25	T	3
11	M	♀ ♀	50	S	3
13	M	♀ ♀	18	T	3
15	M	♀ ♀	19	T	3
16	M	♀ ♀	29	S	3
28	M	♀ ♀	29	S	1
30	M	♀ ♀	18	T	3
33	M	♀ ♀	25	S	3
34	M	♀ ♀	19	T	3
39	M	♂ ♂	42	S	1
41	M	♀ ♀	40	S	1
36	?	♀ ♀	24	S	3

with respect to any factor, the pair was listed as dizygotic. If the co-twins agreed in all blood factors tested, eye and hair color and a qualitative comparison of dermatoglyphic patterns were used to confirm the diagnosis of monozygosity. As a result of these further studies, one pair (No. 37) was classed as dizygotic and another (No. 36) was classed as doubtful. This latter pair is listed in tables 1, 2, and 3, but was not used for the statistical analysis. The diagnostic criteria used in the body build study itself will be given in the report of that study.

We have obtained urine samples from 27 pairs of twins, i.e., from 54 persons. All donors furnished the samples voluntarily, since none of them were hospitalized or confined in institutions. Whenever possible, three morning urine samples were collected from each person, usually on three successive days, and both members of a twin pair furnished the samples on the same days. In some cases, as when the donors came to New York for a visit from elsewhere, we had to be satisfied with a single sample, and this was not necessarily a morning sample. The donors were on self-selected diets, which may be presumed to have been somewhat different for the members of different pairs, and rather more similar for members of the same pair, when the donors lived and took their meals together. The relevant information concerning the twins is summarized in table 1.

Except for pair No. 40, who are Negroes, all other twins are whites. Pairs Nos. 4, 5, 9, 15, 16, 17, 27, 30, 33, 34, 37, 39, and 41 belong to the Jewish ethnic group. None of the twins showed any gross physical abnormalities, and, except for pairs 5 and 29, they were all in apparently good health. In pair No. 5 one member, A, was suffering from severe hypertension and was taking the drug Rauwolfia. In pair No. 29 one member, B, while presently in remission, had severe Thyrotoxicosis from the age of sixteen to nineteen.

TECHNIQUES

In the present study we have followed the techniques of paper chromatography developed for urine and described by Berry and Cain (1951) and by Berry (1953). Some minor modifications are specified below.

The concentrations of all the substances studied on the chromatograms are expressed with reference to the quantities of urine containing known amounts of creatinine. The creatinine contents of the urine samples were determined colorimetrically using sodium picrate (Bonsnes and Taussky 1945).

The amino acids were studied on two-dimensional chromatograms, using amounts of urine containing 40 and 80 micrograms of creatinine. The first solvent was a phenol-salt buffer solution, and the second solvent a 2:6 lutidine-water mixture. The chromatograms were sprayed with a .2 per cent ninhydrin solution in water saturated butanol, and heated for 8 minutes at 100°C. For every urine sample, two or more 40 and two 80 microgram chromatograms, i.e., usually four chromatograms in all, were made. To diminish the experimental variance, we have found it expedient to run the chromatograms of a given kind for all the urine samples from a given pair of twins simultaneously. The 40 and 80 series of chromatograms were, however, made at different times. Occasionally, it was found necessary to desalt certain urine samples due to the distorting effect of their high salt concentration on amino acid separation

for the 80 microgram chromatograms. Desalting was accomplished through the use of a RECO electrolytic desalter. Together with every group of chromatograms containing the urine samples, there were run control sheets with known amounts of certain amino acids (glycine, lysine, alanine, threonine, serine, and valine).

The concentrations of lysine were found to be more reliably determined on unidimensional chromatograms made as follows. Quantities of urine containing 20 micrograms of creatinine were applied to paper, two aliquots from each sample from a given pair of twins being applied to the same sheet. Control solutions of mixtures of amino acids were also applied to the same sheet. A 2:4 lutidine:1% KH_2PO_4:ethanol solution (3:2:1) was used as the solvent, followed by the usual spraying with ninhydrin and heating.

Unidimensional chromatograms were also made with quantities of urine containing 100 micrograms of creatinine. These were placed in butanol-acetic acid-water solvent, and developed with diazotized sulfanilic acid-sodium carbonate reagent (Berry et al., 1951). The same sheet of paper always contained the urines from all the samples from a given pair of twins. Each series of samples was tested twice. There were no controls on these sheets, since the chemical nature of the substances appearing on these chromatograms is not known with assurance.

Quantification was carried out by the method of maximum optical density (Block, 1953) through the use of a Photovolt densitometer. In this technique, as the name implies, only the maximum optical density of the spot is recorded. This is a comparatively rapid method of quantification, and it has been well established that the maximum optical density of a substance is in general proportional to the amount of that substance present.

Berry (1953), as well as Sutton and Vandenberg (1953), used area measurements for the spots on the diazotized sulfanilic acid chromatograms, but we found the two spots which we measured (Rf 90 and Rf 85) to be better evaluated by the same photometric device which was used for the measurement of the amino acids.

THE DATA

The mean maximum optical densities of the spots containing glycine, threonine, alanine, and glutamine in the samples from the persons examined are summarized in table 2. As reported in table 1, the number of samples studied was not the same for all persons; at least two chromatograms were, however, prepared from every sample, so that the means in table 2 are based on at least two observations.

An examination of table 2 shows that the concentration of each of the four amino acids varies quite appreciably in the urine of different persons. Thus, the mean readings for glycine vary from 49 to 139, for threonine from a trace to 33, for alanine from 19 to 73, and for glutamine from 24 to 62. It can easily be seen that the co-twins (A and B in table 2) tend to resemble each other more than do members of different twin pairs. The most striking exception to this rule occurs in twin pair No. 37, where twin B excretes appreciably greater quantities of the amino acids (at least relative to the amount of creatinine excreted) than does twin A.

The fact that the co-twins tend to resemble each other in the excretory patterns suggests, but does not by itself prove, that these patterns are to some extent geneti-

TABLE 2.—MEAN MAXIMUM OPTICAL DENSITIES OF THE GLYCINE, THREONINE, ALANINE AND GLUTAMINE SPOTS IN SAMPLES OF THE DIFFERENT TWIN PAIRS. CHROMATOGRAMS WITH AMOUNTS OF URINE CORRESPONDING TO 40 MICROGRAMS OF CREATININE

The two members of each pair are arbitrarily denoted as twin A and twin B.

Twin Pairs	Zygosity	Alanine		Glutamine		Glycine		Threonine	
		A	B	A	B	A	B	A	B
1	D	45.7	34.7	52.2	55.8	96.8	96.3	26.0	18.3
3	D	36.2	24.6	36.9	27.3	67.8	57.0	13.1	13.0
5	D	46.3	36.7	51.5	46.8	89.7	92.0	20.0	24.0
6	D	66.0	73.3	62.3	58.3	138.0	119.0	20.0	17.3
12	D	34.2	38.6	37.7	34.0	82.5	85.2	8.7	15.2
14	D	37.0	39.0	41.5	39.5	64.0	76.0	8.0	11.0
17	D	26.7	27.1	28.4	33.9	49.0	54.3	6.0	0.0
27	D	27.8	26.4	30.0	30.0	74.0	65.1	11.8	8.2
29	D	36.3	44.0	40.8	43.2	100.3	103.3	10.3	9.7
37	D	35.0	64.0	35.7	55.7	61.0	138.7	8.3	32.7
40	D	29.3	23.7	34.3	31.7	69.0	70.3	15.0	11.7
2	M	25.5	33.5	36.7	38.3	75.5	80.0	14.3	13.0
4	M	37.5	35.8	38.4	34.9	69.6	68.9	11.6	10.7
7	M	34.0	36.7	34.0	34.7	87.3	90.0	10.7	12.0
8	M	36.7	38.5	41.0	42.0	119.5	108.5	9.0	8.7
9	M	36.9	37.8	45.2	41.2	79.0	78.5	18.7	18.8
11	M	24.9	28.2	24.5	25.0	81.2	98.8	8.7	8.7
13	M	29.8	25.2	42.0	35.3	87.8	63.5	11.3	10.0
15	M	19.2	21.5	30.3	29.0	61.8	67.2	8.7	6.3
16	M	34.2	42.8	37.7	43.7	106.5	118.8	9.7	11.0
28	M	24.5	28.5	25.0	35.0	64.0	78.7	12.0	11.3
30	M	46.5	36.8	61.3	56.7	94.0	86.2	28.0	27.0
33	M	35.2	30.5	37.7	33.7	75.2	68.8	13.0	11.7
34	M	38.2	50.5	31.3	46.7	54.8	91.8	8.7	12.7
39	M	47.0	41.0	53.0	44.0	56.0	52.0	15.0	11.0
41	M	55.0	62.0	56.0	60.0	102.0	110.0	16.0	17.0
36	?	63.0	45.3	45.0	49.7	99.0	101.7	10.3	11.7

cally controlled. Such a resemblance would be expected on any one of a number of hypotheses, because the twins resemble each other also in age, in upbringing, and, since most of them live together, also in their diets. The hypothesis of genetic causation leads, however, to the expectation that monozygotic twins should, on the average, resemble each other more than do the dizygotic ones. Whether or not this is the case is not apparent at first glance from the data in table 2. This problem requires a statistical examination of the data.

ANALYSIS OF VARIANCE

The first question to be examined is whether the differences between the averages for the co-twins are as great as or greater than could be expected because of the experimental errors, and because of the variations in the composition of the urine from day to day in the same person. As stated above, we had three samples taken on consecutive days from most donors. Comparison of the samples from the same person

TABLE 3.—THE DAY-TO-DAY VARIANCE (σ_d^2), AND THE INTRA-PAIR VARIANCE (σ_t^2) FOR THE TWINS FURNISHING URINE SAMPLES

Twin Pairs	Zygosity	Alanine		Glutamine		Glycine		Threonine	
		σ_d^2	σ_t^2	σ_d^2	σ_t^2	σ_d^2	σ_t^2	σ_d^2	σ_t^2
1	D	11.5	60.5	17.8	6.70	29.7	0.1	4.6	29.4
3	D	3.0	67.8	6.3	46.1	50.5	58.0	3.2	0.0
5	D	13.6	46.7	8.7	10.9	32.4	2.7	8.5	8.0
6	D	—	26.7	—	8.0	—	180.5	—	3.6
12	D	18.7	9.6	12.5	7.0	23.1	3.8	0.2	21.1
14	D	—	2.0	—	2.0	—	72.0	—	4.5
17	D	1.5	0.1	8.0	15.3	2.9	14.2	0.7	18.0
27	D	7.5	0.9	9.1	0.0	50.1	39.6	1.2	6.3
29	D	25.6	29.4	8.3	2.7	25.4	4.5	1.9	0.2
37	D	4.7	420.5	10.3	200.0	154.9	3016.3	8.9	357.5
40	D	0.8	16.0	7.8	3.4	56.7	0.9	0.2	5.6
2	M	20.7	32.0	20.1	1.4	53.8	10.1	2.1	0.9
4	M	1.5	1.4	4.5	6.2	12.7	0.3	1.0	0.4
7	M	—	3.6	—	0.2	—	3.7	—	0.9
8	M	18.9	1.7	16.7	0.5	9.2	60.5	2.9	0.1
9	M	2.8	0.5	3.4	8.0	18.9	0.2	4.7	0.0
11	M	5.3	5.4	2.4	0.3	55.3	154.5	1.3	0.0
13	M	2.0	10.9	5.4	22.4	29.7	295.9	1.4	0.9
15	M	3.1	2.7	1.2	0.9	8.1	14.3	3.8	2.7
16	M	3.1	37.5	2.3	18.0	10.7	76.0	1.4	0.9
28	M	—	8.0	—	50.0	—	108.1	—	0.2
30	M	5.1	46.8	35.1	10.6	59.5	30.7	7.2	0.5
33	M	1.4	10.9	6.1	8.0	18.1	20.1	0.7	0.9
34	M	79.4	76.0	7.8	118.1	22.3	684.5	0.6	8.0
39	M	—	18.0	—	40.5	—	8.0	—	8.0
41	M	—	24.5	—	8.0	—	32.0	—	0.5
36	?	82.6	156.1	9.7	12.50	11.6	3.6	1.8	0.9

yields a variance which subsumes the effects of the day to day variations and of the experimental errors, the latter dampened by the fact that each sample was examined twice, and that we have used the means of the two determinations in our computations. Dividing this variance by the number of days gives the expected variance of the mean for that twin, due to day to day variation and experimental error. The average of this value for each pair of co-twins is denoted by σ_d^2 in table 3. For comparison, the actual intra-pair variance, σ_t^2 was computed, using the means from table 2. This includes the effect of day to day variation and experimental error as well as the inherent difference between co-twins. This variance would on the average equal σ_d^2 if there were no differences between co-twins, but actually is sometimes greater and sometimes less, because of sampling error. Discounting sampling error, $\sigma_t^2 - \sigma_d^2$ gives a measure of the magnitude of the differences between co-twins. No day-to-day variance can, obviously, be computed for the donors who furnished only a single sample; these donors are marked in table 3 by the sign — in the σ_d^2 column.

In some instances the intra-pair variance is much greater than the day-to-day variance. This situation is expected to occur when the twins differ significantly in their

rates of excretion of certain substances. On the other hand, in many cases the two variances are of about the same magnitude, or even the intra-pair variance is smaller. Here the twins are not appreciably different in the excretion rates of the particular substances. As a matter of fact, as chance would have it, some of the intra-pair variances are surprisingly small compared to the day-to-day variances. We have examined the raw data for the possibility that the members of a twin pair show correlated variations in the excretion rates on the same day, thus tending to even up the averages; we find no warrant in the data for such an assumption.

More informative are comparisons of the mean intra-pair variances with the mean inter-pair variances for the monozygotic and dizygotic twins. The mean intra-pair variance is the arithmetic mean of the σ_i^2 values listed in table 3. The mean inter-pair variances are calculated from the averages for each pair of twins using the data listed in table 2. They are then multiplied by two to make them comparable to the intra-pair variances, since the intra-pair variances are based on means of individuals and the inter-pair variances on means of pairs of individuals. The mean variances, and certain ratios of these variances, are listed in table 4, separately for the dizygotic and for the monozygotic twins.

It can be seen in table 4 that the inter-pair variances are appreciably greater than the intra-pair variances for all four substances and for dizygotic as well as for monozygotic twins. The statistical significance of the greater magnitude of the inter-pair compared to the intra-pair variances can be evaluated by computing ratios of these variances, which are also shown in table 4. The excess of the inter-pair variance is significant in most cases, at the 1 per cent level or better. This is a statistical confirmation of the validity of the statement made above on the basis of a simple inspection of the data in Table 2: the members of a twin pair tend to resemble each other more than do unrelated persons. The biological meaning of this fact is not immediately apparent, because co-twins have similar environments as well as similar heredity.

More important for our purposes is a comparison of the intra-pair variances in dizygotic and in monozygotic twins. If the differences between the excretion rates of

TABLE 4.—MEAN INTER-PAIR AND MEAN INTRA-PAIR VARIANCES AND THEIR RATIOS FOR DIZYGOTIC AND MONOZYGOTIC TWINS

Variance or Ratio	Degrees of Freedom	Alanine	Glutamine	Glycine	Threonine
Inter-pair					
Dizygotic	10	317.5	198.4	975.3	74.7
Monozygotic	14	176.1	182.8	617.2	49.8
Combined	24	235.9	189.3	766.3	60.1
Intra-pair					
Dizygotic	11	61.8	27.5	308.4	35.7
Monozygotic	15	18.7	19.6	99.9	1.65
Combined	26	38.4	22.9	188.1	16.1
Inter:Intra Combined		6.12**	8.27**	4.08**	3.74**
Intra Dizygotic:Intra Monozygotic		3.31*	1.40	3.09*	21.65**

* Significant at the 5% level.
** Significant at the 0.1% level.

a given substance found in the group of dizygotic twins who have furnished the urine samples are genetically conditioned, then members of the dizygotic pairs of twins should, on the average, differ more strongly than do members of the monozygotic pairs. As a result, the mean intra-pair variance for the dizygotics should be greater than the corresponding variance for the monozygotics. The ratios of the two variances are shown in the lowermost line of table 4. Their meaning will be discussed below.

CONTROL DATA

As stated above, the data in tables 2–4 are given in terms of the units of maximum optical density. Controls, which contained known amounts of certain amino acids,

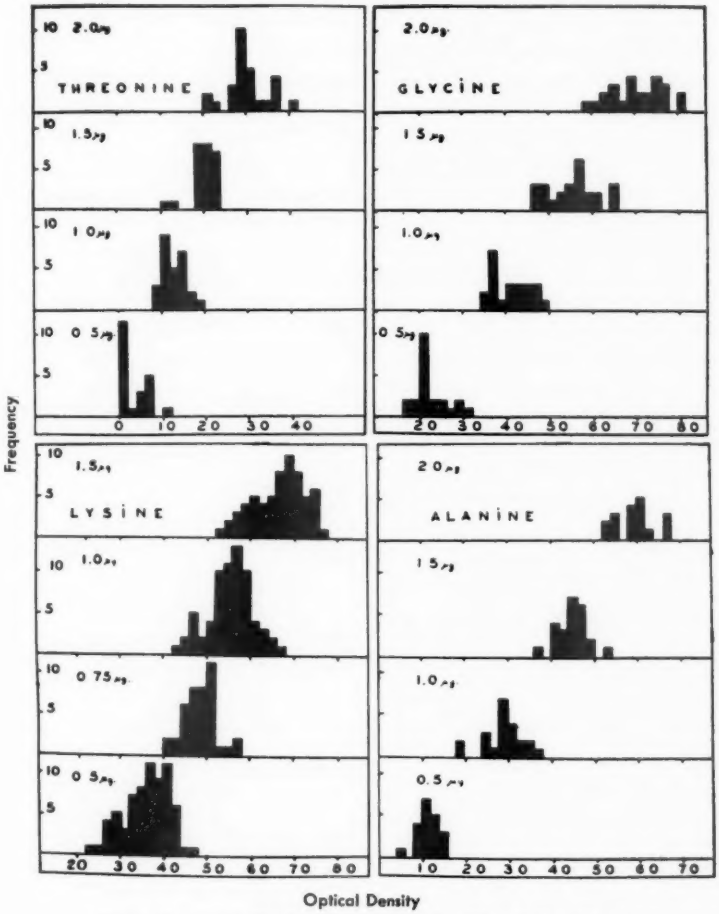

FIG. 1. Frequency distribution of optical densities of control data

TABLE 5.—MEAN OPTICAL DENSITIES FOR CONTROL DATA

	0.5γ	0.75γ	1.0γ	1.5γ	2.0γ
Alanine	11.1		29.1	44.3	61.0
Glycine	21.4		40.1	54.5	69.6
Lysine	36.0	48.6	55.2	66.8	—
Taurine	4.0		11.0	18.0	23.0
Threonine	2.4		11.9	19.0	29.3
Valine	1.9		14.6	28.3	48.6

have been run simultaneously with the urine chromatograms. These control data are summarized, in the form of diagrams, in figure 1. The mean optical densities of the spots on the control chromatograms corresponding to various amounts of the amino acids are given in table 5.

The control data permit the estimation of the magnitude of the experimental errors involved in our measurements. The magnitude of the experimental errors may also be deduced from the experimental data themselves. Indeed, every kind of chromatogram was prepared at least twice for every urine sample studied. Table 6 summarizes the standard deviations for the control measurements, as well as for the replicate chromatograms of the urine samples. Since some urine samples contain much greater quantities of certain substances than other urines, it seemed expedient to compute the standard deviations separately for the samples with high and with low contents of the respective substances. The dividing line between the "Low" and the "High" is, of course, arbitrary. It is given in terms of the units of optical density in the rightmost column of table 5. The most intense spots (those corresponding to alanine, glutamine, glycine, lysine, β-amino-isobutyric acid, and taurine) were measured in chromatograms with amounts of urine containing 40 micrograms of creatinine.

TABLE 6.—STANDARD DEVIATIONS OF REPLICATED DETERMINATIONS OF THE OPTICAL DENSITY OF SPOTS ON CONTROL CHROMATOGRAMS AND ON THOSE WITH URINE SAMPLES

(Further explanations in text)

Substances	Controls					Urine		Dividing Line
	0.5	0.75	1.0	1.5	2.0	Low	High	
Alanine (40)	3.3	—	4.9	3.4	5.3	5.8	5.6	40
DSA—Rf 92	—	—	—	—	—	3.2	4.2	15
DSA—Rf 84	—	—	—	—	—	4.4	6.5	20
Ethanolamine (80)	—	—	—	—	—	6.3	10.6	20
Glutamine (40)	—	—	—	—	—	4.7	6.9	40
Glycine (40)	3.8	—	4.4	5.6	6.1	9.5	11.6	80
Leucine (80)	—	—	—	—	—	1.8	3.3	10
Lysine (20)	5.7	4.2	5.5	6.4	—	3.3	5.0	50
β-amino-isobutyric (40)	—	—	—	—	—	—	3.9	10
Spot No. 22 (80)	—	—	—	—	—	4.4	4.3	10
Taurine (40)	—	—	—	—	—	5.2	8.1	20
Threonine (40)	2.9	—	2.6	3.1	4.9	3.3	4.3	18
Tyrosine (80)	—	—	—	—	—	2.2	3.8	10
Valine (80)	3.3	—	5.5	2.7	6.5	3.8	8.6	20

TABLE 7.—EXCRETION VALUES FOR SOME OF THE AMINO ACIDS STUDIED IN WEIGHT UNITS

Substance	Min.		Max.		Mean		Mean in mg/24 hrs	
	M	D	M	D	M	D	M	D
Alanine	.017	.021	.054	.064	.031	.036	50	58
Glycine	.036	.035	.106	.135	.066	.068	105	110
Lysine	.005	.006	.085	.068	.020	.025	32	40
Taurine	<.012	.014	.100	>.125	.038	.045	61	72
Threonine	.019	<.012	.049	.055	.028	.031	45	50
Valine	<.006	.008	.022	.022	.012	.012	19	19

The less intense spots (ethanolamine, leucine, tyrosine, valine, and others) were measured in chromatograms with the amount of urine containing 80 micrograms.

It can be seen in table 6 that, for most substances, the standard deviations are greater for urines with high than in those with low contents of a given substance. The same will, obviously, be true also for the standard errors. The exceptions from this rule are the substances, such as β-amino-isobutyric acid, which are present in so low a concentration in some urines that they form very faint spots on the chromatograms and cannot be measured reliably.

The control data also permit the translation of some of the optical density readings into weight units of the respective substances. Such data are of value for comparative purposes with other studies. The minima, maxima, and means for the monozygotic (M) and dizygotic (D) twins are given in table 7 below in terms of mg. of substance per mg. of creatinine. For further comparisons the mean values are also given in terms of mg. of substance excreted per 24 hours. This conversion was carried out by assuming a mean 24 hour creatinine excretion of 1600 mg.

DISCUSSION

Twin pair 37 are alike in all blood factors tested, but are classed as dizygotic on the basis of eye, skin, and hair color. Qualitative study of digital patterns puts them in the "doubtful" category. Since excretion patterns are the things being studied, they cannot be used for diagnosis; however, their study in this case strengthens our faith in the diagnosis. In another paper (Gartler, Dobzhansky, and Berry, 1954) it is shown that this pair exhibits a marked difference in the excretion rate of β-amino-isobutyric acid, which has been shown to be under genetic determination (Harris, 1953a, and Calchi-Novati et al., 1953).

The heritability of this substance is not at issue in this paper, and hence it might be used as a diagnostic trait, although it is not as well-established as the blood factors. With respect to the four substances studied in this paper, and some, but not all, of the substances to be reported on later, pair 37 are by far the most discordant of all those studied. Consequently it is of interest to see what the conclusions would be if this pair were excluded. If this is done, the intra-pair variance ratios become 0.38 for glycine, 5.86 for threonine, 1.39 for alanine, and 0.52 for glutamine. The ratio for threonine is still significant (P between 0.1% and 0.5%) but the other three are not significant (P greater than 20%).

In considering whether the results imply heritability, the possibility of environ-

TABLE 8.—AVERAGE INTRA-PAIR VARIANCES FOR TWINS LIVING SEPARATELY AND TOGETHER

	Alanine	Glutamine	Glycine	Threonine
Dizygotics				
Separated	124.0	54.0	652.0	67.0
Separated (Excluding #37)	50.4	17.9	60.3	10.25
Together	9.7	5.0	22.5	9.3
Monozygotics				
Separated	21.8	21.0	42.4	1.9
Together	12.8	19.6	151.6	1.6

mental differences, particularly differences in diet, must be kept in mind. Some light can be thrown on this point by comparing twins living together, and sharing at least two meals a day in common, with twins living apart, and possibly having different diets. For this purpose pair 30, who live together but whose samples were not obtained on the same days, are omitted. Table 8 gives the average intra-pair variances. For the identical twins there is no evidence of any greater variance for twins living apart. For fraternal twins, on the other hand, the variance is far greater for those living apart. Pair 37 happen to live apart, but it is unlikely that this causes their large differences. However, even if they are omitted, fraternal twins living apart have a much larger variance than those living together for three of the four substances, but not for threonine. The reason for the difference between fraternal and identical twins with respect to the effect of living apart is not clear. It might, of course, be due to chance and to the small size of our sample when broken up into four groups instead of two. On the other hand, it might be that identical twins tend to choose more similar diets than fraternal twins, even when living apart. The similarity of fraternal twins, living together and apart, for threonine would then indicate that excretion of this substance was less under environmental control. This would imply that the difference of the intra-pair variance of identical and fraternal twins for threonine was genetically controlled.

We thus see that on the basis of our data there is good evidence that the excretion of threonine is under the control of one or more genes which are segregating in our material. On the other hand there is only weak and unsatisfactory evidence for the heritability of alanine and glycine, and none at all for glutamine.

Sutton and Vandenberg (1953) found that siblings are significantly more similar than non-siblings in threonine excretion, while no significant differences appeared for glycine or for alanine. To this extent, our results and those of Sutton and Vandenberg reinforce each other. On the other hand, Sutton and Vandenberg found the siblings to resemble each other also in glutamine excretion, while our data for glutamine are negative. It should be noted that on the basis of the comparison of inter-pair and intra-pair variances, which was used by Sutton and Vandenberg, all four substances are highly significant for our monozygotic and dizygotic twins combined. However, for our data similarity of environment for co-twins might explain this. It is also conceivable that similarity of early environment for Sutton and Vandenberg's sib pairs might explain part of their results, even though all their subjects were in the more or less uniform environment of an institution at the time of the study.

On the other hand, even if the difference between our conclusion and that of Sutton

and Vandenberg with respect to glutamine should be due to differences in heritability, this would not necessarily be a contradiction; it would be naive to conclude from our data, or the data of the authors just cited, either that the glutamine excretion ratios are "inherited" or that they are "not inherited". What the data really show is that, among the dizygotic twins who furnished the urine samples which we studied, there were some pairs the members of which were genetically different in their threonine excretion rates. It happened that these twins were either genetically alike in glutamine excretion, or else the differences were too small to produce statistically significant results.

A "trait" is an abstraction used for descriptive purposes; what we actually observe are individual organisms which go through certain processes of living and development, and in so doing acquire certain "traits" of form and function. In a sense, any "trait" is inherited, since there must be a body to show the "trait", and there must be a genotype to engender the development of the body. Genetics as a science is concerned not with "traits", but with differences between the developmental patterns of individuals and populations which result in perceptible variations in visible or measurable "traits". The fact that neither our data, nor the data of Sutton and Vandenberg, reveal genetic differences in the excretion rates of glycine and alanine certainly does not mean that these rates are not under genetic control. Indeed, the excretion rates of these substances are sharply increased by certain pathological conditions some of which are known to be genetic. Perhaps the best known of such conditions is the Fanconi syndrome, which is apparently caused by homozygosis for a single recessive gene (Bickel and Harris, 1952). It would be by no means unexpected if also non-pathological genetically conditioned variations in the excretion rates of glycine and alanine were discovered in some populations.

The problem at issue in this investigation has already been stated in the Introduction. It is simply that of the heritability of the variations in the excretion rates of various substances observed in a given sample of the population. The evidence presented indicates some heritability of the variations in the threonine excretion, but suggests that the heritable variations in the excretion of the other three substances are, if they exist at all, so rare or so small that they are marked by environmentally induced variations. Data bearing on the heritability of variations in the excretion rates of other substances in the urine will be presented in the following paper.

SUMMARY

The objective, methods, material, and part of the results of a study of urinary excretion patterns in twins are described in this paper. A twin study was used in order to obtain some idea as to whether the variability in the chromatographically determined excretion rates of the different substances studied had a genetic basis. The results of four of the substances investigated (alanine, glutamine, glycine, and threonine) are included in this paper. A comparison of the intra-pair variances of excretion rates for monozygotic twins compared with those for dizygotic twins indicates that the variation in the excretion rate of threonine is at least partially under genetic control. However, the evidence is negative for the excretion rate of glutamine and ambiguous at this time for alanine and glycine.

ACKNOWLEDGMENTS

The authors wish to thank Mrs. T. D. Gidaspow who assisted in various technical aspects of the work and performed the calculations. Our thanks are also due to the Rockefeller Foundation for financial support of this work. Dr. F. J. Kallmann was the sponsor of one of us (S. M. Gartler) during his tenure of a U. S. Public Health Service Post-doctoral Fellowship. Professor Roger Williams, of the University of Texas, kindly extended the hospitality of his laboratory to one of us (Th. Dobzhansky) to learn the techniques of the paper chromatography, as applied especially to urine and as developed by his collaborators. Above all, we owe our thanks to the twins whose cooperation made this study possible.

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Chromatographic Studies on Urinary Excretion Patterns in Monozygotic and Dizygotic Twins

II. Heritability of the Excretion Rates of Certain Substances

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INTRODUCTION

HUMAN URINE is a remarkably complex mixture of substances. Some of these substances, such as urea and creatinine, are excreted by all humans, the quantities eliminated being relatively simple functions of the body mass and of the intensity of the tissue metabolism. Other substances are excreted after certain foods are ingested, and do not appear in the urine with other diets. An example of such a substance is methyl histidine, which forms a greenish grey spot on chromatograms developed with ninhydrin, and which may be present in greatly varying amounts in urine samples taken from the same person on successive days. Datta and Harris (1951) have shown that methyl histidine is excreted in urine following consumption of meat. This is understandable since methyl histidine is a breakdown product of anserine, which is a constituent of vertebrate muscles. Still other substances are present in the urine of most persons, but are produced in highly varying amounts, without an obvious relation to diet. Here belong some of the free amino acids, which are present in the urine in small but not negligible amounts (of the order of one to two per cent of the total nitrogen excreted). These amino acids are filtered by the renal glomeruli, but mostly reabsorbed in the renal tubules. The reabsorption is, however, incomplete, and a part of the amino acids is passed into the urine. The amounts so eliminated may vary depending either on the concentration of the respective substances in the blood, or on the height of the renal threshold, or on both factors. Both the variation in the concentration in the blood and in the renal thresholds is in some cases known to be genetically determined, and in other cases due to environmental agents.

The three classes of urinary constituents indicated above are obviously not sharply distinct from each other. Nevertheless, it is the third class that seems most promising for genetic study. We have made paper chromatograms of samples of urine of 27 pairs of twins, using in part phenol-lutidine and in part butanol-acetic acid solvents, and ninhydrin and diazotized sulfanilic acid as developers (see the foregoing paper by Berry et al., 1954). About forty different kinds of spots appear on such chromatograms, some visible because of their color in ordinary light and others fluorescent in ultraviolet light (Williams and collaborators 1951), some present in all or most urine samples and others in a minority of them, some representing known chemical substances and others not yet chemically identified. In the foregoing paper the varia-

Received September 14, 1954.

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tions in the excretion rates of glycine, threonine, alanine, and glutamine have been discussed. Certain other substances will be considered in the following pages.

THE DATA

The data are presented in the same manner as was done in the preceding article (Berry et al. 1954) with all the signs and symbols having the same meaning as they had in that paper. Consequently, the presentation of the data will not be explained again here.

In table 1 are given the mean maximum optical densities of the spots containing β-amino-isobutyric acid, ethanolamine, leucine, lysine, taurine, tyrosine, valine and unidentified substances designated #22, Rf 84, and Rf 92 in the urine samples from the persons examined. For further details see the preceding paper. The day-to-day variation (σ_d^2) and the intra-pair variance (σ_t^2) for all twin pairs examined are given in table 2. The mean day-to-day variances and the mean intra-pair variances for the dizygotic and monozygotic twins are given in table 3 along with the inter-pair variances for the two kinds of twins. The ratios of intra-pair variances for dizygotics to monozygotics and their significance levels are also given.

β-AMINO-ISOBUTYRIC ACID

Harris (1953) has shown that the variations in the concentration of this substance in human urine are genetically conditioned, high concentrations being apparently due to homozygosis for a single recessive gene. This was confirmed by Calchi-Novati et al. (1953) on the basis of a study of 152 families with 407 children. The above authors used chromatograms with quantities of urine considerably smaller than ours, and they found the β-amino-isobutyric acid spot strongly expressed in only a minority (between 5 and 10 per cent) of the urine samples. With amounts of urine containing 40 or 80 micrograms of creatinine, traces of this spot are found in practically all samples, although the measurements of the optical density of the weaker spots are often handicapped by the proximity of the methyl histidine spot. For purposes of calculation, we have given the value of zero to all β-amino-isobutyric acid spots which produced readings of less than 6 units on the densitometer. As a consequence, 23 of the 54 individuals studied are recorded to have average values of 0 for this substance, although most of them do have traces of it visible at least on the 80 microgram creatinine chromatograms (table 1). Among the remaining individuals, 22 have average values for the spot below 20, 6 have averages between 20 and 30, and 3 above 30. These 3 individuals are probably the "positives" of Harris and of Calchi-Novati et al.

It can be seen (table 2) that the day-to-day variances are much smaller than the intra-pair variances in the dizygotic twins, but usually not in the monozygotic ones (except No. 15). The mean day-to-day variance is about ten times smaller than the mean intra-pair variance for the dizygotics, but it is of the same order of magnitude for the monozygotics (table 3). In other words, the differences between the members of the monozygotic pairs are only about as large as are the day-to-day variations, while the differences between dizygotic twins are often much larger (cf. twins Nos. 1, 5, and 29, table 2).

TABLE 1.—MEAN MAXIMUM OPTICAL DENSITIES OF CERTAIN SPOTS ON THE URINE CHROMATOGRAMS OF TWIN PAIRS

Chromatograms with Amounts of Urine Corresponding to 20, 40, 80, and 100 Micrograms of Creatinine have been used.

Twin No. and Zygosity	β-Amino Isobutyric (40)	Ethanol amine (80)	Leucine (80)	Lysine (20)	Taurine (40)	Tyrosine (80)	Valine (80)	Spot # 22 (80)	Rf 84 (100)	Rf 92 (100)
1A,D	14.7	21.7	18.3	57.0	23.5	8.3	18.0	15.3	11.3	31.3
1B,D	32.7	18.3	18.7	40.0	21.8	6.0	17.3	15.7	25.7	49.5
3A,D	0	37.3	15.3	37.7	25.1	2.0	8.7	4.3	10.7	22.2
3B,D	0	28.3	9.0	22.3	18.3	2.7	9.0	6.0	10.7	15.0
5A,D	20.3	—	21.3	37.3	19.2	15.7	15.3	13.3	0	32.0
5B,D	4.7	29.3	22.2	51.7	32.5	10.7	13.8	13.7	7.0	22.0
6A,D	0	18.0	22.0	87.0	25.3	14.0	10.0	3.0	21.5	26.0
6B,D	0	0	21.0	55.0	14.0	22.0	39.0	19.0	11.0	26.0
12A,D	0	13.2	17.2	36.0	14.0	6.0	11.7	7.0	14.7	18.5
12B,D	0	14.7	14.7	54.0	8.4	4.0	15.2	4.2	21.4	23.2
14A,D	4.0	18.0	12.0	45.0	35.5	13.0	10.0	5.0	7.0	24.0
14B,D	0	16.0	15.0	29.0	25.5	0	12.0	3.0	9.5	17.0
17A,D	4.7	18.3	10.7	55.0	10.7	1.0	11.3	7.7	16.2	22.7
17B,D	0	22.0	8.7	53.0	7.0	9.0	7.7	9.7	14.2	20.7
27A,D	2.9	35.3	17.3	53.0	19.1	10.0	11.7	11.7	22.8	24.2
27B,D	0	27.7	12.0	58.0	14.4	5.3	17.7	9.0	22.0	18.0
29A,D	15.0	16.0	18.0	47.7	18.5	14.7	17.3	15.3	24.2	23.2
29B,D	2.3	31.3	19.3	39.7	36.5	7.3	12.3	12.0	13.0	15.7
37A,D	25.7	13.2	15.2	66.5	27.0	8.7	17.2	16.5	18.7	58.7
37B,D	10.0	5.2	15.7	82.0	56.3	7.3	15.2	13.8	29.3	18.0
40A,D	0	32.3	10.3	66.3	19.3	0.7	7.0	6.0	9.5	23.5
40B,D	0	24.7	15.7	62.7	13.7	0	8.3	10.3	7.7	34.0
Average.....	6.2	20.6	15.9	51.6	22.1	7.6	13.9	10.0	14.9	25.7
2A,M	2.5	18.7	9.0	28.0	29.0	1.0	10.0	13.0	12.0	22.0
2B,M	1.5	18.3	7.3	21.7	19.5	0	8.3	10.0	13.3	20.0
4A,M	0	13.3	12.3	31.7	17.9	1.0	6.0	4.0	15.8	20.8
4B,M	2.4	10.3	11.3	33.3	17.1	1.0	7.3	1.3	12.3	23.7
7A,M	0	—	9.0	36.0	48.7	6.0	5.0	2.0	7.0	23.0
7B,M	0	—	11.0	38.0	45.3	7.0	2.0	5.0	5.5	18.0
8A,M	51.8	0	15.5	39.7	7.3	0.7	38.0	18.5	8.0	13.0
8B,M	53.7	0	11.5	50.0	3.3	0	30.5	14.0	8.0	12.7
9A,M	0	14.5	10.2	59.7	32.0	0	6.7	8.0	7.6	10.5
9B,M	1.4	22.2	11.2	57.0	40.7	0	7.7	7.8	6.5	12.0
11A,M	15.4	7.7	10.3	20.0	7.3	0	12.7	14.3	11.0	17.3
11B,M	13.8	2.0	11.0	31.3	5.7	0	19.0	13.3	13.2	17.7
13A,M	1.7	12.7	13.3	49.0	15.7	2.3	8.0	10.3	10.2	18.7
13B,M	0	13.7	13.3	45.3	5.3	5.7	8.7	9.7	15.3	18.2
15A,M	27.5	32.0	12.3	51.7	5.3	0	8.0	1.3	9.0	11.3
15B,M	20.7	34.3	13.3	49.3	15.0	0	9.3	3.0	10.0	16.7
16A,M	14.8	22.0	15.0	56.3	24.3	16.0	19.7	16.0	11.0	18.8
16B,M	14.0	5.3	17.3	57.3	13.7	20.0	24.3	20.0	12.5	19.0
28A,M	0	0.0	15.0	36.0	12.7	5.5	12.5	7.0	23.5	20.0
28B,M	0	36.5	9.0	46.0	0	10.0	8.0	3.0	19.0	28.5
30A,M	0	—	21.3	116.3	43.7	15.3	19.3	20.0	15.7	10.3
30B,M	0	—	26.0	102.7	40.0	12.0	18.7	16.0	12.7	8.0
33A,M	4.8	4.3	11.3	53.0	11.3	5.7	13.3	11.3	27.3	21.7

TABLE 1.—*Cont.*

33B,M	4.3	4.3	10.0	47.7	23.3	4.0	11.0	7.3	23.2	17.5
34A,M	26.8	0	14.0	61.0	9.7	6.7	12.7	15.3	36.5	26.3
34B,M	22.5	1.7	15.3	68.3	13.3	15.7	13.3	16.0	25.2	24.5
39A,M	0	41.0	15.0	58.0	7.0	10.0	6.0	10.0	8.0	23.0
39B,M	0	45.0	12.0	60.0	6.0	11.0	8.0	11.0	14.0	29.5
41A,M	4.0	9.0	18.0	48.0	0	14.0	34.0	18.0	11.5	28.5
41B,M	7.0	16.0	19.0	58.0	0	13.0	37.0	17.0	10.0	22.0
Average.....	9.7	14.8	13.4	50.3	17.4	6.1	14.2	10.8	13.8	19.1
36A,?	2.3	29.5	19.2	49.0	30.7	18.5	11.2	7.8	27.3	26.0
36B,?	0	23.3	20.3	51.5	13.0	19.8	15.5	11.2	22.0	38.0

A strong indication of a high degree of heritability of the excretion rates of the substance under consideration can be seen in that the mean intra-pair variance (σ_i^2) for the dizygotic twins is about 15 times greater than that for the monozygotics. Such a variance ratio (F value) is expected to arise by chance in less than 1.0 per cent of trials. It may also be noted that the mean inter-pair variance (σ_p^2) is very much greater than the intra-pair variance for the monozygotic, and only about three times as great for the dizygotic twins (table 3).

The data of Berry (1953), of Sutton and Vandenberg (1953), as well as our own, show that all concentrations of β-amino-isobutyric acid, from a trace to very high, occur in different persons. This fact does not contradict the existence of a single major gene determining the excretion rate of this substance, as inferred by Harris and by Calchi-Novati et al. (see above). Our data suggest, however, that, apart from this gene, there may exist other genetic modifiers of the excretion rates.

ETHANOLAMINE

This substance forms an intense spot on the chromatograms of some urine samples, but seems to be wholly absent in others (table 1). It shows also great day-to-day variations in the urine samples from the same person, the average day-to-day variance being of the same order of magnitude as the average intra-pair variance in dizygotic, but smaller in monozygotic twins (table 3). The mean inter-pair variances are, however, considerably greater than the mean intra-pair variances.

The available data are most compatible with the assumption of a low heritability, but a high environmental modifiability, of the excretion rates of ethanolamine. It must, however, be noted that the measurements of the optical density of the ethanolamine spot are not satisfactory on some chromatograms, which have this spot streaked towards the valine spot above it. Examination of table 1 shows indications of a negative correlation between the concentrations of ethanolamine and valine in our data, which may mean that under some conditions ethanolamine migrates further upwards and fails to separate from valine.

LEUCINE

In our material, almost all the chromatograms made with quantities of urine containing 80 micrograms of creatinine show a leucine spot, albeit a very faint one in

TABLE 2.—THE DAY-TO-DAY VARIANCE (σ_d^2) AND THE INTRA-PAIR VARIANCE (σ_t^2) FOR THE TWINS FURNISHING URINE SAMPLES

Twin Pairs	Zygosity	β-Amino-Isobutyric		Ethanolamine		Leucine		Lysine		Taurine		Tyrosine		Valine		Spot #22		Rf 84		Rf 92	
		σ_d^2	σ_t^2	σ_d^2	σ_t^2	σ_d^2	σ_t^2	σ_d^2	σ_t^2	σ_d^2	σ_t^2	σ_d^2	σ_t^2	σ_d^2	σ_t^2	σ_d^2	σ_t^2	σ_d^2	σ_t^2	σ_d^2	σ_t^2
1	D	14.7	161.5	12.4	5.6	8.8	0.1	48.5	144.5	23.1	1.4	1.7	2.7	6.1	0.2	0.4	0.1	8.2	102.8	121.7	165.1
3	D	0.0	0.0	5.3	40.5	0.9	20.0	6.1	117.7	8.8	23.1	4.1	0.2	10.9	0.1	3.4	1.4	0.2	0	2.6	25.7
5	D	10.4	122.6	—	—	8.5	0.4	69.8	102.8	29.2	88.9	5.4	12.5	10.2	1.1	2.0	0.1	12.2	24.5	—	50.0
6	D	0.0	0.0	—	162.0	—	0.5	—	512.0	—	63.8	—	2.0	—	420.5	—	128.0	—	55.1	—	0.0
12	D	0.0	0.0	32.1	1.1	0.7	3.1	11.4	162.0	3.6	15.8	2.0	2.0	3.0	6.1	5.8	3.8	16.4	22.0	26.3	11.3
14	D	—	8.0	—	2.0	—	4.5	—	128.0	—	50.0	—	84.5	—	2.0	—	2.0	—	3.1	—	24.5
17	D	4.1	10.9	51.1	6.7	1.1	2.0	6.2	2.0	23.2	6.7	0.7	32.0	7.8	6.7	0.9	2.0	15.1	2.0	1.8	2.2
27	D	1.8	4.2	4.4	29.3	2.9	14.2	14.7	12.5	68.8	10.9	9.6	10.9	0.9	18.0	5.9	3.6	15.8	0.3	29.7	19.0
29	D	8.1	80.3	9.7	117.5	1.4	0.9	6.0	32.0	14.7	162.0	2.9	26.9	4.4	12.5	2.2	5.6	7.9	62.4	10.1	28.1
37	D	1.4	122.8	1.7	32.0	3.0	0.1	42.7	120.1	129.1	430.1	0.4	0.9	7.0	2.0	1.2	3.6	1.8	56.9	24.2	827.0
40	D	0.0	0.0	113.0	29.3	4.4	2.7	49.8	6.7	17.2	16.0	0.2	0.2	3.1	0.9	8.9	9.4	1.3	1.7	13.6	55.1
2	M	1.9	0.5	59.3	0.1	2.1	1.4	115.3	20.1	16.3	45.1	0.5	0.5	15.7	1.4	2.3	4.5	3.6	0.9	9.0	2.0
4	M	0.8	3.0	14.1	4.5	1.9	0.5	13.1	1.4	4.8	0.3	1.0	0.0	2.9	0.9	3.1	3.6	10.6	6.1	16.3	4.3
7	M	—	0	—	—	—	0.5	—	2.0	—	5.8	—	0.5	—	4.5	—	4.5	—	1.1	—	12.5
8	M	17.9	1.7	0.0	0.0	7.2	8.0	26.6	53.4	2.4	8.0	0.2	0.2	151.6	28.1	14.6	10.1	0.0	0.0	5.4	0.1
9	M	0.1	1.0	12.8	30.0	0.7	0.5	103.5	3.8	18.6	37.8	0.0	0.0	1.48	0.5	0.8	0.0	1.5	0.6	6.8	1.1
11	M	6.2	1.2	4.7	16.1	2.2	0.2	32.4	64.2	13.3	1.2	0.0	0.0	21.9	20.0	0.8	0.5	6.2	2.4	26.6	0.1
13	M	1.4	1.4	45.6	0.5	2.8	0.0	25.6	6.7	6.3	53.5	1.1	5.6	0.9	0.2	4.8	0.2	2.5	13.3	2.8	0.2
15	M	3.6	23.3	6.6	2.7	2.3	0.5	10.3	2.7	41.6	46.8	0.0	0.0	1.1	0.9	1.8	1.4	5.0	0.5	0.9	14.3
16	M	1.7	0.3	28.8	138.9	2.6	2.7	12.0	0.5	30.4	56.8	0.0	8.0	11.8	10.9	0	8.0	3.8	1.1	25.7	0.0
28	M	—	0.0	—	666.1	—	18.0	—	50.0	—	80.6	—	10.1	—	10.1	—	8.0	—	10.1	—	36.1
30	M	0.0	0.0	—	—	8.9	10.9	344.4	93.3	9.7	6.7	0.9	5.5	3.1	0.2	14.3	8.0	2.1	4.5	11.4	2.7
33	M	1.7	0.1	11.0	0.0	1.0	0.9	8.1	14.2	11.9	72.0	1.9	1.4	1.6	2.7	1.1	8.0	42.4	8.7	2.5	8.7
34	M	8.9	9.4	0.4	1.4	3.7	0.9	32.2	26.9	3.8	6.7	0.8	40.5	1.9	0.2	6.2	0.2	5.6	64.2	4.3	1.7
39	M	—	0.0	—	8.0	—	4.5	—	2.0	—	0.50	—	0.5	—	2.0	—	0.5	—	18.0	—	21.1
41	M	—	4.5	—	24.5	—	0.5	—	50.0	—	0.0	—	0.5	—	4.5	—	0.5	—	1.1	—	21.1
36	?	0.7	2.7	36.6	19.0	33.5	2.2	38.1	3.1	45.6	156.1	18.0	0.9	0.8	9.0	3.1	5.8	2.9	14.2	16.7	72.0

TABLE 3.—MEAN DAY-TO-DAY VARIANCE (σ_d^2), MEAN INTRA PAIR VARIANCE (σ_t^2), INTER-PAIR VARIANCE (σ_p^2) AND INTRA-PAIR VARIANCE RATIOS FOR DIZYGOTIC AND MONOZYGOTIC TWINS

Substance	Dizygotic			Monozygotic			σ_t^2 Dizygotic
	σ_d^2	σ_t^2	σ_p^2	σ_d^2	σ_t^2	σ_p^2	σ_t^2 Monozygotic
β-Amino-isobutyric	4.50	46.38	139.18	4.02	3.10	434.54	14.9***
Ethanolamine	28.71	42.61	147.22	18.34	68.68	246.48	0.6
Leucine	3.52	4.41	29.74	3.22	3.34	29.48	1.3
Lysine	28.34	121.85	384.58	65.77	26.08	825.24	4.6***
Taurine	35.31	78.98	111.56	14.48	28.13	403.58	2.8*
Tyrosine	3.00	15.90	49.26	.58	4.89	72.84	3.2*
Valine	5.94	42.74	45.20	19.45	5.81	189.28	7.4***
Spot #22	3.43	14.49	33.33	4.53	3.87	64.36	3.7**
Rf 84	8.77	30.08	81.96	7.56	8.85	69.94	3.4*
Rf 92	28.75	109.82	113.00	10.14	8.40	57.10	13.0***

*** Significant at less than the 0.5% level.
** Significant at less than the 1.0% level.
* Significant at less than the 5.0% level.

some instances (table 1). The measurement of the optical density may be interfered with by the proximity of the phenylalanine spot, but it can be made accurately if proper care is exercised. Berry (1953) found that leucine (and ethanolamine) give some of the lowest ratios of inter-person to day-to-day variances. We find the day-to-day variances to be about as great as the intra-pair variances, both for dizygotic and for monozygotic twins (table 3). The mean intra-pair variance for the dizygotics is little greater than for the monozygotics. Our data give no evidence of any appreciable degree of heritability of the excretion rates of leucine.

LYSINE

The measurements of the optical density of the lysine spot are difficult in some two-dimensional chromatograms because of a diffuseness of this spot. More satisfactory measurements are obtained on uni-dimensional chromatograms prepared with amounts of urine containing 20 micrograms of creatinine, and treated as described in the preceding paper of Berry et al. The data for lysine in tables 1–3 are based on readings made in such uni-dimensional chromatograms. With this technique, we find measurable amounts of lysine in all urine samples which we have studied.

Berry (1953) found lysine to give one of the lowest ratios between the day-to-day and the person-to-person variances among the substances which she has studied. In our material, the mean intra-pair variance is 4.3 times greater than the mean day-to-day variance for the dizygotic twins, while for the monozygotics this ratio is 0.4. More important still, the mean intra-pair variance for the dizygotics is 4.6 times greater than that in monozygotics (table 3).

A rather high degree of heritability of the observed variations in the excretion rates of lysine can be inferred from the data. Indeed, several dizygotic twin pairs are markedly discordant as to the lysine excretion (see twin Nos. 1, 3, 5, 12, 14, 37, and especially 6, tables 1 and 2). The day-to-day variations in the excretion of lysine

are, nevertheless, considerable. Berry (unpublished) found lysine excretion to vary particularly in connection with different stages of the menstrual cycle and of pregnancy. It should be noted that Sutton and Vandenberg (1953) found no evidence for the heritability of the excretion rates of lysine in their work.

TAURINE

The concentration of taurine varies greatly from day to day in the urine of the same person, and even more in different persons. This substance has been found to be lacking almost entirely in the twin pair No. 41, but it gives one of the most prominent spots in the chromatograms of the urine of some other persons (see table 1). The mean intra-pair variance for the dizygotic twins is only 2.8 times as great as that for the monozygotics (table 3). Berry (1953) and Sutton and Vandenberg (1953) also found little indication of heritability of the excretion rates of this substance.

TYROSINE

Tyrosine forms one of the less conspicuous spots on chromatograms developed with ninhydrin. This spot, which is brownish instead of purple in color, is absent or too weak to give a reliable measurement in about one-third of the urine samples in our material (about one-half according to Berry, 1953). Nevertheless, the data suggest that the excretion rates of tyrosine are to some extent under genetic control.

The day-to-day variance of tyrosine excretion is in general low (except the twin pair No. 27, table 2). The mean intra-pair variance is appreciably greater than the day-to-day variance; the inter-pair variance is 15 times as great as the mean intra-pair variance in monozygotic and 3 times as great in dizygotic twins (table 3). The mean intra-pair variance for dizygotic twins is 3.2 times greater than in the monozygotics. Such an F value is likely to occur by chance in only about 2 per cent of trials.

VALINE

This substance forms a perceptible spot in most chromatograms with amounts of urine containing 80 micrograms of creatinine. Unfortunately, the measurements of the optical density of this spot may be handicapped by the failure of separation from ethanolamine (see above).

The mean intra-pair variance for valine is some 7.4 times greater in dizygotic than in monozygotic twins (table 3). This would seem to indicate a rather high degree of heritability. However, examination of the data shows that the high mean intra-pair variance for the dizygotic twins is caused largely by the strong discordance in the twin pair No .6, where one of the twins excretes a great amount of valine while the other twin is average or even under average in excretion of this substance (tables 1 and 2).

SPOT #22

A ninhydrin-positive substance of unknown chemical composition forms a purplish spot on two-dimensional chromatograms made with phenol and lutidine at a place above and to the left of β-amino-isobutyric acid (see Figure 1 on page 75 in Williams and collaborators, 1951). The spot #22 appears on most chromatograms

of urine samples, albeit its intensity varies greatly from person to person (table 1). The mean day-to-day variance is about as great as the mean intra-pair variance for monozygotic, but appreciably smaller in dizygotic twins (table 3). The ratio of the intra-pair variances in dizygotic and monozygotic twins is 4.3, which is significant statistically at the 1 per cent level. It must, however, be noted that this rather high F value is due, like that for valine, chiefly to the discordance in the twin pair No. 6 (tables 1 and 2). The heritability of the excretion rates of the substance under consideration must remain in doubt pending further work.

GLUTAMIC ACID

Berry (1953) found measurable amounts of this substance in the urines of 166 out of the 255 adult persons which she studied. In our material, glutamic acid is recorded in the monozygotic twin pairs No. 7 (optical densities 18 and 34 respectively in the single samples from the two twins), No. 8 (optical densities 22–28 in one, 12–30 in the other twin), No. 16 (0–32 in one, 6–24 in the other twin), No. 33 (0–14 in both twins), and No. 34 (12–24 in one, zero or trace in the other twin). Among dizygotic twins, the glutamic acid spot appears in both twins No. 5 (16–30 in one, 14–28 in the other), in both twins No. 35 (8–38 in one, 0–32 in the other), and in No. 6 (0–8 in one, 42–44 in the other). The large discordance among the monozygotic twins No. 34 argues against the variations in the excretion rates of glutamic acid being strictly heritable.

SERINE

This amino acid forms one of the relatively prominent spots on most chromatograms of urine samples. Unfortunately, with the technique used by us, the serine spot is adjacent to that of glycine, and this makes the photometric measurement of serine unreliable in samples having large amounts of glycine.

PROLINE

This substance gives a yellow spot on chromatograms sprayed with ninhydrin. Its occurrence in more than trace amounts is, however, infrequent. In our material proline is definitely present in the single sample from one of the twins of the dizygotic pair No. 6, but absent or present as only a trace in the other twin. Proline is also recorded in one, but not in the other, twin of the pair No. 3, who are dizygotic, and in both monozygotic twins No. 15. The data thus suggest that the excretion of amounts of proline perceptible with our technique is heritable, but more information is needed to establish this point.

THE SPOTS #29, 31, 32, AND 42

These ninhydrin-positive substances are known only by the position of the spots which they form on two-dimensional phenol-lutidine chromatograms (see Figure 1 on page 75 in Williams and collaborators, 1951). The intensity of these spots is in general low, hence they were recorded in chromatograms containing amounts of urine with 80 micrograms of creatinine.

The spot #29 is rather conspicuous in the urine samples of both twins of the dizygotic pairs Nos. 27 and 35. It is also present in both twins of the pairs Nos. 11 and

36. In the dizygotic pair No. 29, one of the twins has this spot very clear, while the other lacks it entirely or shows only a trace of it.

The spot #31 is present in both twins Nos. 2, 39, and 40. Nos. 2 and 39 are monozygotic, and No. 40 are dizygotic. It is very suggestive that one of the dizygotic twins No. 1 shows this spot very clearly (optical density 18–36), while the other twin seems to lack it entirely. A similar discordance is observed among the dizygotic twins Nos. 3 and 37. Curiously enough, this spot has not appeared in measurable intensities in any of the monozygotic twins.

The spot #32 is clearly present only in both twins No. 40 (dizygotic). The spot #42 is recorded in both twins No. 6 (dizygotic) and No. 41 (monozygotic). Any inferences about the heritability of these rare spots would be clearly premature at present.

SPOT RF 84

This is a bright orange spot appearing on chromatograms treated with the butanol-acetic acid solvent and sprayed with diazotized sulfanilic acid. Both Berry (1953) and Sutton and Vandenberg (1953) measured the area of this spot on the chromatograms; Berry found the inter-person variance to be 4.9 times greater than the day-to-day variance, while Sutton and Vandenberg found that siblings resemble each other more closely than non-siblings with respect to the size of this spot. We have measured the intensity of the Rf 84 spot photometrically, rather than by its area.

The mean day-to-day variance is approximately equal to the mean intra-pair variance for monozygotic twins; for the dizygotics, the latter variance is 3.4 times greater than the former (table 3). The variance ratio for the intra-pair variances in the dizygotic:monozygotic twins is 3.4, which is significant at the 2.5 per cent level. Reference to tables 1 and 2 shows that several dizygotic twins are appreciably discordant, while among the monozygotics only the pair No. 34 is rather discordant. Finally, the inter-pair variances are 8 times greater than the mean intra-pair variance for monozygotic, and 2.7 times greater for dizygotic twins (table 3). All the evidence thus agrees that the excretion rates of the chemically unknown substance forming this spot are to some extent under genetic control.

SPOT RF 92

The chemically unknown substance forming this spot on chromatograms treated with the butanol-acetic acid solvent and sprayed with diazotized sulfanilic acid behaves very much like the substance forming the spot Rf 84 (see above). The spot Rf 92 is, however, deep purple in color. We have measured it chromatographically, while Berry (1953) and Sutton and Vandenberg (1953) used area measurements. Berry found the inter-person to day-to-day variance ratio to be 4.7, but Sutton and Vandenberg did not find a significantly greater similarity between siblings than between non-siblings.

Our data show the spot Rf 92 present on most chromatograms. The day-to-day variations are quite large; the average day-to-day variance is of the same order of magnitude as the mean intra-pair variance in monozygotic; but about ¼ as large in dizygotic twins (table 3). The mean intra-pair variance for dizygotic twins is 13

times greater than that for the monozygotics. Such an F value is likely to occur by chance in less than 0.1 per cent of trials. Furthermore, examination of table 2 shows that sizeable intra-pair variances have been observed in several of the dizygotic twins, and in only one monozygotic pair (No. 28, for which only a single sample is available). The large value of the intra-pair variance for dizygotic twins is due largely to pair No. 37, who are for many substances our most discordant pair; however, even if they are omitted, the mean intra-pair variance for dizygotic twins is 4.5 times that for monozygotic twins. Such an F value would arise by chance less than 1% of the time. The evidence thus indicates that excretion of the substance composing this spot is subject to genetic control.

THE DAY-TO-DAY VARIANCES

Examination of table 3 discloses that the average day-to-day variances for taurine, tyrosine and Rf 92 are significantly greater for fraternal than they are for monozygotic twins, while for lysine and valine they are significantly smaller in the dizygotic twins. For the remaining substances the day-to-day variances are about equal for both groups of twins. Now there is no reason to suppose that the excretion pattern in an individual is affected by the existence of a twin, whether mono- or dizygotic. As pointed out to us by our colleague, Howard Levene, this peculiar situation is probably due to a departure of the observed distribution of the excretion rates from normality. In point of fact, some individuals show very large day-to-day variations in the excretion rates of certain substances (e.g., the twins 2, 9, and 30 for lysine, table 2). Such a lack of normality should result in about as many F ratios significantly large as significantly small, and this is what happens for the ratios of day-to-day variances, and intra-pair to day-to-day variances for monozygotic twins. On the other hand this ratio for dizygotic twins is always greater than one. This indicates real differences for the dizygotic twins for at least some of these substances, though it might not be too obvious for just which ones. Furthermore, both here and in the inter-pair vs. intra-pair comparisons, the differences, even though proved, could conceivably, although not likely, be due to environmental factors rather than heredity.

VARIANCES IN TWINS LIVING TOGETHER AND TWINS LIVING APART

Ideally, twin studies should be made placing the mono- and the dizygotic twins in similar and uniform environments. Indeed, the use of the variance ratios implies the assumption that the environmental conditions for the monozygotic co-twins are as similar as those for the dizygotic co-twins. Only in this case does a greater discordance among dizygotic than among monozygotic twins prove a genetic determination of the observed variability. For obvious practical reasons, the above ideal is rarely if ever attained in actual work. As a consequence, one must carefully examine the data for the possible disturbing effects of environmental variations. In our material, such examination discloses a most interesting, and possibly significant, circumstance.

As shown in table 1 of the foregoing paper by Berry et al., some of the co-twins in our material resided and took their meals together, while other co-twins lived

TABLE 4.—INTRA-PAIR VARIANCES AND VARIANCE RATIOS FOR DIZYGOTIC AND MONOZYGOTIC TWINS LIVING TOGETHER [T] AND APART (S)

Substance	Dizygotics		Monozygotics		σ_t^2 Dizygotics (T) / σ_t^2 Monozygotics Pooled
	$\sigma_t^2 T$	$\sigma_t^2 S$	$\sigma_t^2 T$	$\sigma_t^2 S$	
Lysine	57.2	199.4	20.1	23.6	2.3
Taurine	42.6	95.6	20.7	36.6	1.5
Tyrosine	26.1	3.7	5.8	2.2	7.4*
β-amino-isobutyric	17.2	81.4	5.1	0.9	5.5*
Valine	7.7	84.8	6.9	4.3	1.4
#22	4.4	26.6	2.6	4.3	1.3
Rf 84	15.3	47.9	11.0	6.7	1.7
Rf 92	23.4	213.6	4.3	14.8	2.8

* Significant at less than the 1% level.

and took their meals separately. We may, then, inquire whether the common residence and diet produce appreciable reductions of the intra-pair differences between the co-twins. The intra-pair variances for the twins living together and apart are given in table 4. For the monozygotic pairs, the variances for the twins living together and apart are mostly of the same order of magnitude (except for the spot Rf 92, for which the variance for the twins living separately is about three times greater than that for the twins living together). The situation is quite different in dizygotic twins. Here the variances are greater in the twins living apart for every substance studied except one (tyrosine). Examination of table 3 in the paper of Berry et al., and of table 2 in the present article, also shows that high intra-pair variances occur mostly in the dizygotic twins living apart. In other words, monozygotic twins living separately still remain fairly similar in the excretion rates of most of the substances studied, while among dizygotic twins separate residence causes appreciable discordance in the excretion rates of the same substances.

Superficially considered, the above fact may seem to indicate that the greater variances observed for dizygotic than for monozygotic twins are due to environmental differences. The rightmost column in table 4 shows ratios of the intra-pair variances for dizygotic twins living together to the corresponding variances for monozygotic twins regardless of common or separate residence. These variance ratios are statistically significant only for β-amino-isobutyric acid and for tyrosine. However, the situation is in reality more complex and more interesting than it may at first appear. Indeed, the monozygotic twins living apart do not show increased intra-pair variances (except for Rf 92). The most likely, although not conclusively proven, explanation of this fact is that monozygotic twins tend to select more or less similar environments, and particularly similar diets, even when living apart, while dizygotic twins do not show such similar choice as frequently.

In order to evaluate this fact properly in terms of the heritability of the excretion rates of the substances studied, one must keep in mind that what is transmitted by

heredity is not an excretion rate as such, but a pattern of environmental responses which, under certain conditions, results in certain excretion rates. The environmental responses include, of course, the choice of the environments and diets by the persons concerned. The proximate cause of the greater intra-pair variance among the dizygotic twins living apart is, then, environmental, but, since the choice of different environments is genetically conditioned, the ultimate cause is genetic. Such a genetic mechanism may, conceivably, produce differences in the excretion rates of certain substances even if the reabsorptive capacities of the renal tubules were alike for these substances. In reality, it is most probable that the two factors which bring about the differences between individual excretory rates interact, the factor of choice being more important for some substances and the factor of renal threshold for others. But this is a matter which concerns the developmental mechanisms which bring about phenotypic differences in the excretion rates, rather than the degree of heritability of these excretion rates. We may be dealing here with a situation which illustrates nicely the dynamic nature of the process of heredity. This is a matter for further research.

DISCUSSION

The variability among humans of the excretion rates of certain substances in the urine constitutes a most attractive, and yet scarcely explored, field of genetic study. The potential importance of the work in this field may be very considerable. Discovery of new heritable traits in man is always interesting, but this may prove to be the less important aspect of genetic studies on the metabolic variation in our species. Metabolic variants are likely to influence the adaptive values of their carriers in certain environments in a more immediate and clear fashion than do the variations in most morphological traits studied by anthropologists and geneticists. Some of the known metabolic variations are rare and occur as pathological aberrants; others are common, and constitute a part of the normal polymorphism of human populations. Some of the pathological aberrants have been carefully studied by physiologists and biochemists; these studies may prove very helpful in understanding the physiological basis also of the normal polymorphism.

As is perhaps natural for studies in a vast and novel field, the work on metabolic variability is beset with many difficulties. For the time being, the greatest of these is technical. This is the lack of precision in the measurement of the concentrations of various substances in the urine samples. The high variability observed in our control data (table 5 in the foregoing paper of Berry et al.) shows that paper chromatography, as we have used it, yields only quasi-quantitative results. However, the more refined chemical and microbiological methods that are available for certain substances found in the urine would not solve our difficulty, and this not only because most of these methods are prohibitively laborious. The fact never to be lost sight of in genetic studies on the metabolic variations is that the excretion rates of some of the substances which may prove most interesting to a geneticist vary quite appreciably from day to day in the same person. Such variations have been found by Berry (1953) and Sutton and Vandenberg (1953) for several amino acids, the daily excretion rates varying in some instances by a factor of two in the same person. Such variations were observed also by Denko and Grundy (1949) for tryptophane.

The reality of the day-to-day variations in the excretion rates of certain substances may be inferred also from our data. Since each urine sample obtained from the twins has been chromatographed twice, we have a measure of the magnitude of the experimental error involved in replicate tests on the same sample. On the other hand, the mean values for the different samples produced by the same person on successive days may be compared. There is no doubt that the day-to-day variations can not be accounted for by the experimental errors alone. This is perhaps what is expected since urine excretion functions, in part, as a homeostatic mechanism which maintains a constant internal environment in the organism in the face of dietary and other environmental fluctuations.

Very little is known about the environmental variables affecting amino acid excretion. However, as was mentioned in the previous paper, a certain amount of work has been carried out on the effect of varying dietary protein on amino acid excretion. The results of these studies are not always unambiguous, but taken together they leave no doubt that the excretion rates of at least some amino acids are influenced by varying protein intake.

However that may be, the day-to-day variations in the excretion rates are so great that any high precision analytical techniques would be in a large measure wasted when applied for study of single urine samples. The problem of genetic study of the excretion rates is nevertheless not insoluble, owing to the fact that the person-to-person variations in the excretion rates are so great that both the experimental errors and the day-to-day variations are dwarfed for at least some of the substances (see table 5 in Berry 1953, tables 3 and 4 in the foregoing paper of Berry et al., and tables 2 and 3 of the present paper).

It is most likely that the observed variations in the excretion rates are both under genetic and under environmental control. This is what is expected on theoretical grounds for traits that are adaptively important and that involve functions which must function differently in different environments (cf. Dobzhansky and Wallace, 1954). The interplay of genetic and environmental factors may furnish valuable experimental approaches to the study of the role of the metabolic variations in the evolutionary processes taking place in our species. It is, on the whole, unlikely that traits of this sort will often be found governed by clear-cut gene differences with major effects, such as those displayed in blood antigens. The normal polymorphism in metabolic traits in human populations is in all probability under polygenic control. Furthermore, there is the remarkable fact that dizygotic twins living apart show significantly greater variances for the excretion rates of certain substances than do dizygotic twins living together, while no such relationship is observed among monozygotic twins. This fact may conceivably give an indication of the adaptive mechanisms connected with the genetically controlled excretion rates.

SUMMARY

Excretion rates of certain substances, chiefly amino acids, have been compared in dizygotic and monozygotic twins. The data presented in this paper, and in the foregoing paper of Berry et al., indicate a participation of genetic factors in the control of the excretion rates of β-amino-isobutyric acid, threonine, tyrosine, lysine, and an

unknown substance designated Rf 92. Less conclusive is the evidence of heritability of the excretion rates of alanine, glycine, taurine, valine, #22, Rf 84, and proline. The data are negative for ethanolamine, glutamine and leucine. Dizygotic twins living apart show greater intra-pair variances for the excretion rates of several substances than do dizygotics who live together. No such relationship is, however, observed for monozygotic twins living apart or together.

ACKNOWLEDGMENT

The writers wish to acknowledge their obligations to Professor Howard Levene for many discussions of the data and of the interpretations contained in this paper, and to Mrs. T. D. Gidaspow who has carried out the necessary calculations.

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The Inheritance of Personality

A Multiple Variance Analysis Determination of Approximate Nature-Nurture Ratios for Primary Personality Factors in Q-Data

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I. THE MULTIPLE VARIANCE ANALYSIS DESIGN

WITH INCREASING appreciation in psychology of the importance both for theory and practice of greater functional understanding of the role of heredity in abilities and personality development, including abnormal personality development, the problem of nature-nurture research techniques has recently been attacked with renewed vigor. The research now to be described differs in three significant ways from any previous work in the field.

1. It uses a multiple variance analysis design, recently described and discussed (8), in the place of the usual comparison of identical with fraternal twins. (1) (2) (11) (20) (24) (25) (27) (31) (36).
2. It deals with *dimensions* of personality which have been established by *factor analytic* investigations upon personality responses in rating data, questionnaire data, and objective tests. (6) (9) (19).
3. It estimates the reliability of measurement of the *essential factor* and works out the nature-nurture ratio for this factor rather than for the tests themselves.

To put this research approach in perspective in regard to other studies, it is necessary first to point out that it follows one of the two chief *possible* avenues, the other being the approach by Mendelian ratios, which is not adapted to most normal psychological dimensions, because they are, presumably, multi-gene determined. Within this aim of nature-nurture ratio determination, previous work has examined just three dimensions, namely, general mental capacity, (1) (2) (24), rigidity or perseveration (11) (36), and general neuroticism (18). General ability has been studied extensively (28), but a few names, such as those of Newman, Freeman and Holzinger (24) and Burks (2) stand out in decisive studies. Their work has been judiciously appraised by Schwesinger (28), Thorndike (33) and Woodworth (35) and recently confirmed by Blewett (1). The inheritance of rigidity was examined some years ago by Yule (36) and, along with fluency, by Cattell and Malteno (11), but inconclusively; while an exploratory study of the inheritance of neuroticism has recently been reported by Eysenck and Prell (18). Practically all of these studies used the identical twin-fraternal twin comparison, and did not adequately distinguish between the ratio for the test measurements and the ratio for the implied factor.

The present study is considered still to be at the exploratory level. For, introducing

Received July 7, 1954.

as it does a new method, and entering a factor domain in which less than one-sixth of the dimensions here investigated have been previously studied, it could and does encounter some difficulties. Because of this exploratory nature of the design, we have felt justified in working with the most convenient and brief experimental device, namely the questionnaire. The Q-data thus obtained, however, can be related to behavior ratings and objective personality tests through previous researches (6) (12) (16) integrating factor measures from different media. Accordingly, we regard our findings as initial, approximate statements of these important nature-nurture ratios, which are to be followed by more exact values based on objective test measurements, made with whatever methodological improvements are developed through the present exploratory study. The nature and meaning of the individual personality factor measurements and the nature-nurture ratios will be examined after we have set out the basic design, in the present section, as it applies to the factors generally.

What we have called the Multiple Variance Analysis Design (8) must be clearly distinguished in objectives and design from the typical analysis of variance design, though it is related to it in so far as it deals with analysis-of-variance principles. It differs in that it attempts to determine ratios for hypothetical contributions, and in that it is not concerned primarily with a significance test but with determining a *quantitative value for a ratio.* In the previous article on this design (8), we have pointed out that the hitherto prevalent method of comparing differences between identical twins with differences between fraternal twins has several weaknesses (25). First, it does not give us all the ratios in which we are interested when we are dealing with the practical psychological problems of everyday life. In particular it fails to give the ratio of between-family variance from environmental causes and between-family variance from hereditary causes. This is one of the most important ratios in many clinical and social calculations. Secondly, it is unable to estimate or allow for the correlation between within-family variance due to heredity and within-family variance due to environment. Thirdly, it makes the assumption, which every psychologist knows to be false, that the particular within-family variance due to environment which it determines—namely, that of twins—is the same as the general within-family variance (of sibs) due to environment. Actually, the differences of environment between siblings, born at different periods in the family life and not reacted to as "twins" are, is bound to be greater than this determination would indicate.

Multiple variance analysis was described by the senior author as a new method in the original article (8) but it has since transpired that although it is new to psychology something closely similar has been independently developed in certain agricultural researches (3) (17) (22) (26). However, the latter have commonly been considered as *ad hoc* extensions of analysis of variance and have apparently not explicitly developed the methodological statement that (a) ratios rather than significance tests *per se* are sought (b) hypothetical variances not directly measurable constitute the concepts under consideration (c) these are reached not by experimentally holding constant all but the required variance, but by solving a sufficiency of simultaneous equations. In the present, psychological data the aim is to solve both for

variances and correlations, and to test the significance of the magnitudes of the ratios obtained for the hypothetical, contributory, environmental and hereditary, intra- and interfamilial variances.

II. THE ELEVEN OR FIFTEEN POSSIBLE SIMULTANEOUS EQUATIONS

In our original statement of the multiple variance design we restricted ourselves to the use of five or six equations, but in our further explanations of the design it became evident that we could get eleven equations, or fifteen if we gathered data also on half-sibs. For reasons explained later we cannot solve for eleven unknowns by the eleven equations, but since other researchers might find them useful we shall set them all down. The remaining four equations for half-sibs, on which we have no data, will be merely listed, without discussion. In each case the value given on the left is an experimentally obtained variance. In our studies it can be and is corrected for error by subtracting from it the error variance, which is a function of the test reliability (general consistency coefficient, specific to the sample). The values on the right are the hypothetical constituent hereditary and environmental variances.

It has seemed best to us to arrange the equations with the first six dealing with variance among individuals and the later equations with variance among families. However, the reader will notice that another arrangement must also be kept in mind, namely, the supplementary relations between "within" and "between" family variances for the same types of 'family', which would indicate that we adjoin equations 3 and 7, 4 and 8, 5 and 9, 1 and 10, and 2 and 11.

1. $$\sigma^2_{ITT} - (1 - r_{ITT})\,\sigma^2_{ITT} = \sigma^2_{we'}$$

This indicates that the differences between identical twins squared, summed and divided by $2n$, (n being the number of pairs), can be considered as due to the within-family environmental difference in the factor being measured, e.g., in intelligence, plus the error of measurement in the test. It will be noticed that the *we* has a prime upon it, indicating that it is different from the more common within-family environmental difference[1]—*we*—where ordinary *siblings* are involved. (ITT = Identical twins raised together.)

2. $$\sigma^2_{FTT} - (1 - r_{FTT})\,\sigma^2_{FTT} = \sigma^2_{wh} + \sigma^2_{we'} + 2r_{wh.we'}\sigma_{wh}\sigma_{we'}$$

This states that the variance among fraternal twins reared together (*FTT*) is equal to the usual inter-sibling hereditary variance, which we shall in general call the within-family hereditary variance, and which is due to the differential segregation of the parental genes, and the within-family environmental variance, in this case again differentiated as that peculiar to fraternal twins. Since the environmental effects may be correlated with the hereditary effects within the family, in that a child showing a certain hereditary endowment may get treatment different from one

[1] The environmental variance, within families, among twins, must also include that *among twin pairs* (when more than one pair of twins are considered typical of a "twin family"). When we pair sibs at random the intra-pair differences include the equivalent of this difference. For simplicity of representation we shall assume that we' does also, though we could split it into two terms.

with a different hereditary endowment, we must introduce the term $2r_{wf.we'}\sigma_{wf}\sigma_{we'}$ on the assumption that this r cannot be treated as zero. (However, we are compelled later to treat $r_{wh.we'}$ and $r_{wh.we}$ as identical).

The present equations are, of course, statements of expectation, on theoretical grounds, about the hypothetical variances that will contribute to an observed variance, and about their modes of combination. In both respects there are five shades of difference of possible assumption into which we do not have space to enter here. For example, we have differentiated σ^2_{we}, $\sigma^2_{we'}$ and $\sigma^2_{we'}$ where some investigators might not think it necessary, and in the later half-sib equations we have assumed that mother and father contribute equally to hereditary variations where a finer distinction might take into account the X chromosome. The principle assumptions that the reader might wish to debate, however, are those concerning covariances or correlations that may or may not be considered zero among variances. Our procedure has been to consider all the *mathematically possible* combinations of the variances that enter a given equation and to reject immediately, at this first stage, those which, on analysis of variance principles, could not enter into the final variance. At a second stage, *after* setting out the present equations, we shall argue that some of the correlation terms must be, on general biological and social grounds, absolutely trivial and that there is no reason for retaining them. Thirdly, when we combine these "cleaned up", *scientifically* appropriate equations and find we cannot get an algebraic solution without a further assumption about correlation terms, we shall proceed to *approximate* solutions by dropping some more (or assuming fixed values for them). These three stages in the form of the equations should be realized in the subsequent developments.

3. $$\sigma^2_{ST} - (1 - r_{ST})\,\sigma^2_{ST} = \sigma^2_{wh} + \sigma^2_{we} + 2r_{wh.we}\sigma_{wh}\sigma_{we}$$

This expression, for siblings reared together (ST), is just the same as the previous one except that it has the full range of intra-familial environmental variance—*we*.

4. $$\sigma^2_{SA} - (1 - r_{SA})\,\sigma^2_{SA} = \sigma^2_{wh} + \sigma^2_{we} + \sigma^2_{be} + 2r_{wh.we}\sigma_{wh}\sigma_{we}$$

This refers to the formula for siblings reared apart from birth in different families. It should be noted that within-family environmental variance must still be introduced since the brothers are not only in different families but may occupy different positions in the families into which they are adopted. It is conceivable that a prime could be added to *we* and *be* here, on the assumption that foster homes have a slightly different within and between-family environment al range than normal families, but we have approximated and simplified by rejecting this assumption.

5. $$\sigma^2_{UT} - (1 - r_{UT})\,\sigma^2_{UT} = \sigma^2_{wh} + \sigma^2_{we} + \sigma^2_{bh} + 2r_{wh.we}\sigma_{wh}\sigma_{we} + 2r_{wh.bh}\sigma_{wh}\sigma_{bh} + 2r_{we.bh}\sigma_{we}\sigma_{bh}$$

This is the variance for unrelated children reared together. Again it will be noted that the within-family variance term must enter, (in this case the within-family *hereditary* variance). It enters because although they are from different families they present different possibilities in the hereditary compositions derivable from the

parents of that family. Thus, these children possess both the between-family heredity and the within-family heredity variance. Being brought up in the same family they do not have the between-family environmental variance.

Unrelated children could include the situation where all are also unrelated to the acting parents, or where all but one are unrelated, but we have aimed at the first situation. In neither case, however, is there any ground for expecting a relation between *bh* and *wh*, so we can say even at this stage that the fifth term on the right would vanish, and it will so be omitted from later equations.

$$6.\quad \sigma^2_{UA} - (1 - r_{UA})\,\sigma^2_{UA} = \sigma^2_{wh} + \sigma^2_{we} + \sigma^2_{bh} + \sigma^2_{be} + 2r_{we.wh}\sigma_{we}\sigma_{wh} + 2r_{be.bh}\sigma_{be}\sigma_{bh}$$

This is the variance for unrelated children reared apart. That is to say, we are dealing with the variance for people taken at random from the general population. Naturally, it includes all four sources of variance, but, as argued in (5), drops *bh.wh* and *be.we* terms.

Next we begin a series of five between-family variances corresponding to the within-family variances of equations 1 through 5. The individual measures on which these are based are not, it is true, experimentally independent of those in equations 1 through 5; but, in terms of *sampling*, the within and between variances are independent (have independent distributions and are uncorrelated) and hence are usable, for example, as independent estimates of the total population variance. Strictly, these "variances" are "mean squares" rather than variances. For it is simplest in conception and computation to deal with equations using terms of between family *differences* and the resulting totals are twice the variance of family means and half the variance of family sums. Thus the corresponding "within" and "between" variances mentioned above—equations 3 with 7, 4 with 8, 5 with 9, 1 with 10 and 2 with 11—sum to *twice* the total population variance actually obtained directly—that in equation 6. This follows in accordance with the analysis of variance model, when we take $k - 1$ degrees of freedom for k families in calculating the between variance, and $km - k$, (where m is 2—the two sibs or twins) for the within variance. However, the difficulties we later encounter in the use of these ensuing equations have nothing to do with this trivial "catch", but arise from interaction of the systematic paired relationships of equations just discussed. The result is, that except where some special correlation of variance term breaks the relation, the later equations are obtainable as linear combinations of these earlier ones. They thus fail to be algebraically usable as additional equations permitting solutions for additional terms. However, they will now be stated, because they can nevertheless be used in different combinations and for a least squares solution.

$$7.\quad \sigma^2_{BNF} - (1 - r_{BNF})\,\sigma^2_{BNF} = 2\sigma^2_{bh} + 2\sigma^2_{be} + 4r_{bh.be}\sigma_{bh}\sigma_{be} + \sigma^2_{we} + \sigma^2_{wh} + 2r_{we.wh}\sigma_{we}\sigma_{wh}$$

The expression *BNF* means "between natural families" and is obtained from the differences of the means of pairs of siblings in different natural families from the mean of all such families. This can be obtained by taking the same experimental data as is used in equations 1, 2, and 3, above.

8. $\sigma^2_{BBF} - (1 - r_{BBF})\,\sigma^2_{BBF} = \sigma^2_{we} + \sigma^2_{wh} + \sigma^2_{be} + 2\sigma^2_{bh} + 2r_{we.wh}\sigma_{we}\sigma_{wh} + 4r_{we.bh}\sigma_{we}\sigma_{bh}$

Just as the values in 7 were obtained by taking inter-familial differences from experimental data already used in equation 3, so here we obtain inter-familial differences from the same data that is used in an intra-familial sense in equation 4. Siblings reared apart belong to the same biological hereditary family. Hence the expression *BBF* means "between biological families", i.e., true families which are socially dispersed. It should be noted that the coefficient of the last term would need to be modified from 4 to 2 if we dealt with situations where one child is raised in its own family and one in a foster family. For in the former case there could be no covariance of *we* and *bh*, since *bh* is constant as *we* varies. However, we completely avoided such cases.

9. $\sigma^2_{BSF} - (1 - r_{BSF})\,\sigma^2_{BSF} = \sigma^2_{we} + 2\sigma^2_{be} + \sigma^2_{bh} + \sigma^2_{wh} + 2r_{we.wh}\sigma_{we}\sigma_{wh} + 2r_{we.bh}\sigma_{we}\sigma_{bh} + 2r_{wh.bh}\sigma_{wh}\sigma_{bh}$

Similarly, this takes the inter-familial variance for the data analyzed intra-familially in equation 5. That is, it takes the differences of mean among families that are merely *social families*, consisting of adopted children from hereditarily different families, gathered within the same social families. As in 5 we can decide straightway that $r_{wh.bh}$ is essentially zero and that the last term will therefore disappear from subsequent calculations.

10. $\sigma^2_{BITF} - (1 - r_{BITF})\,\sigma^2_{BITF} = \sigma^2_{we'} + 2\sigma^2_{wh} + 2\sigma^2_{be} + 2\sigma^2_{bh} + 4r_{be.bh}\sigma_{be}\sigma_{bh}$

This is the variance among the means of identical twin families. It uses a specific within-family environmental variance but assumes nothing specific about the between-family heredity of twin families.

11. $\sigma^2_{BFTF} - (1 - r_{BFTF})\,\sigma^2_{BFTF} = \sigma^2_{we''} + \sigma^2_{wh} + 2\sigma^2_{be} + 2\sigma^2_{bh} + 4r_{be.bh}\sigma_{be}\sigma_{bh} + 2r_{we.wh}\sigma_{we}\sigma_{wh}$

This repeats 10 for fraternal twin families. It should be noted that though these formulae distinguish the *we* variance of sibs, identical twins and fraternal twins there seems no reason to multiply unknowns by assuming that the *correlation* of environment and heredity is systematically different in the first and last cases.

It should be noted that the reliabilities in inter-familial variances are the reliabilities for the *mean* score of two sibs or twins.

For completeness we next add four equations, the data for which are obtainable (but not obtained in this research) from families of half-sibs, namely, the children of different fathers born to and reared by one mother, and children of the same father born to and reared by different mothers. Other combinations of birth and rearing are conceivable, but might be far too rare to permit a sufficient sample.

12. $\sigma^2_{HST} - (1 - r_{HST})\,\sigma^2_{HST} = \tfrac{1}{2}\sigma^2_{bh} + \sigma^2_{wh} + \sigma^2_{we} + 2r_{wh.we}\sigma_{wh}\sigma_{we}$

HST equals half-sibs reared together.

13. $\sigma^2_{HSA} - (1 - r_{HSA})\,\sigma^2_{HSA} = \tfrac{1}{2}\sigma^2_{bh} + \sigma^2_{wh} + \sigma^2_{we} + \sigma^2_{be} + 2r_{wh.we}\sigma_{wh}\sigma_{we} + r_{bh.be}\sigma_{bh}\sigma_{be}$

This applies to half-sibs apart, typically the same father and different mothers.

14. $\sigma^2_{BHST} - (1 - r_{BHST})\,\sigma^2_{BHST} = \tfrac{3}{2}\sigma^2_{bh} + \sigma^2_{wh} + 2\sigma^2_{be} + \sigma^2_{we} + 3r_{bh.be}\sigma_{bh}\sigma_{be} + 2r_{we.wh}\sigma_{we}\sigma_{wh}$

BHST means among means of pairs of half-sibs reared together, i.e., the supplement of (12).

15. $\sigma^2_{BHSA} - (1 - r_{BHSA})\,\sigma^2_{BHSA} = \tfrac{3}{2}\sigma^2_{bh} + \sigma^2_{wh} + \sigma^2_{be} + \sigma^2_{we} + 3r_{be.bh}\sigma_{be}\sigma_{bh} + 2r_{we.wh}\sigma_{we}\sigma_{wh}$

The assumption in the last four equations is that father and mother contribute equally to the child's heredity, but the existence of the *X*-chromosome makes this an approximation.

The writers wish to express their great indebtedness to Professor C. R. Rao of the Indian Statistical Institute, Calcutta, for help freely and generously given in searching out errors in the writer's original equations, for improvements, and for proposals for overcoming difficulties in the subsequent solutions.

In principle, the eleven equations offer a complete solution, providing we agree to consider the within-family environmental variance of identical twins to be the same as that of fraternal twins, i.e., $we' = we''$, which is no great assumption, since many parents do not know whether their twins are identical or fraternal. There would then be eleven unknowns: five unknown variances, σ^2_{we}, $\sigma^2_{we'}$, σ^2_{be}, σ^2_{bh} and σ^2_{wh}; and six unknown correlations, as listed in the first article (8).

Unfortunately there is many a slip between the ideal possibility and what can actually be done with a given set of equations. The afore-mentioned linear dependencies, and certain peculiarities of the quadratics, considerably restrict our power of solution. The adaptions made to these restrictions require a section on their own, and are therefore postponed to the computational account in Section 4, while we turn to the psychological measurement data itself.

III. THE Q-DATA PERSONALITY MEASUREMENTS FROM THE J.P.Q. TEST

As indicated above, the only source traits or basic dimensions of personality that have so far received genetic study are those of general mental capacity, rigidity and general neuroticism. The second is not known to be a general *personality* factor, though it was once thought to be, and still apparently remains, a real factor in a certain area of *test* response (6). Although the two true dimensions indicated (intelligence and neuroticism) received prior attention because they were isolated early and assumed to be of greatest practical social and clinical importance, it is now evident that they are no more or less important, in terms of life criteria, than the more recently established personality dimensions. At present, some fifteen or sixteen primary personality factors have been isolated and confirmed, in behavior rating or *L*-data (6) (9), in questionnaire responses or *Q*-data (14) (15), and in objective, behavioral, test responses or *T*-data (9) (10). The matching of factors among the three

media is not complete (6) (9) (12) (16) (19), nor do we yet know the significance of some of these factors in various clinical, scholastic, and general life situations. But, we do know that these factors, based on a thorough sampling of behavior, (6), constitute the greater part of the framework upon which a systematic knowledge of personality can be built by further research. Wherever exact measurement and an objective scientific search for laws has proceeded it has encountered these factor structures, and as personality study advances beyond qualitative, philosophical and clinical speculation, it is likely to organize itself increasingly about these factors as functional unities. Accordingly, we have made them the center of our genetic study, in order that the genetic findings may adhere to psychological measurements of permanent value, and also in order that, reciprocally, psychological theory about the nature of the factors may be guided by the first available genetic information.

It is not claimed in principle, nor will it subsequently transpire in fact, that our research clears up the issues as completely as our theoretical approach might suggest. For the geneticist, our contribution is largely methodological, revealing what sizes of sample, reliabilities of measurement, etc., are necessary to give answers of a given accuracy with the multiple variance analysis method. However, although the answers on the specific psychological data do not have the accuracy that the geneticist would require for a "content" contribution, they still offer a real contribution to the psychologist's world, because the latter has been *completely* in the dark regarding the degree of inheritance of personality factors. Even the present conclusions, with their broad margin of standard error, can help the psychologist a lot in choosing directions of valid hypothesis formation for these primary personality factors. For example, it is a waste of experimental effort to explore a physiological, genetic theory of a personality factor if that particular factor proves in our results to be largely environmentally determined. Conversely, much sociological and learning theory "explanation" has already been embarrassingly committed regarding factors which are here shown, whatever the exact figures, to be at least determined substantially by genetic variance.

In short, a proper strategy of investigation of personality source traits requires that the nature-nurture ratios be determined *as soon as measurement of the factors has reached a stage where even a tolerable reliability of measurement exists*. Psychological research of the last fifteen years has succeeded primarily in *demonstrating the factor patterns* as such, i.e., as invariant, replicatable, loading constellations from sample to sample. Research has only in the last two or three years turned its attention to developing reliable batteries for measuring these factors (10) (15), to permit the present crucial research.

Admitting, therefore, that the time is not ripe to enter this experiment with expectations of establishing the nature-nurture ratios with *exactness*, we yet assert it is important for the orientation of immediate research that a substantial hint be obtained, as early as possible, as to whether a factor is largely determined by environmental molding influences or by constitution. Accordingly, this research has been undertaken as soon as: (1) it is possible to set up testing batteries; (2) of fair validity and reliability, (3) on an array of factors that have so far been shown to be invariant. A practical difficulty here has been that we can best work with *children*

in genetic investigations whereas the advance of factor test measurement has been greatest in the *adult* range and it must be reiterated that we cannot measure these factors in children with the accuracy of, say, intelligence. However, the work of Cattell and Gruen, (14) (16), Beloff (15), Dubin (9), and others in the last few years has provided a reasonably good basis for a child battery, in terms both of questionnaire tests and of objective tests.

The present report restricts itself to the results of the questionnaire battery measurements. These are of lesser validity but greater convenience than the apparatus tests, and it was felt that to operate first with these would give preliminary results and also smooth out the difficulties of the research design for the ensuing objective test battery. This laboratory had recently finished research on the only existing completely factored questionnaire test for children, *The Junior Personality Quiz*, measuring twelve personality factors, one of them being intelligence (15). This test takes less than an hour to administer (¾ of an hour for average subjects) and is only 144 items in length, which means that it proceeds with the maximum validity and reliability now obtainable from 12 items per factor measurement. It has the advantage that most of its factors can be matched with those of the 16 Personality Factor Questionnaire (13), which is a widely used questionnaire test with adults, and that they are also recognizable in terms of the corresponding behavior rating factors, (16) (19).

The factors measured by the J.P.Q. are those of Cyclothymia vs. Schizothymia (Factor A), Neuroticism vs. Ego-Strength (Factor C), Dominance vs. Submissiveness (Factor E), Surgency vs. Desurgency (Factor F), General Intelligence (Factor B), Tender-minded vs. Tough-minded (Factor I), Nervous Tension vs. Autonomic Relaxation (Factor Q4), as well as the factors known as D, H, J, K, and Q_3. The test is designed in the reading vocabulary of an eleven to fifteen year old child. With the indicated brevity of 12 items for each factor the reliabilities of factor measures range from .25 to .83 with a median at .51. Since the construction of the test (15) is such as to avoid any common factors, other than the primary factor concerned, in the halves of the factor measure, the internal validities of the tests, in terms of the extent to which they measure the factor back of the actual questions, are the square roots of these values, and accordingly range from .50 to .91 with a median value of .71. The Junior Personality Quiz is a new test which has been widely used in the first year of its existence, but because of its newness the results of such use have not yet appeared in terms of publication of *specific* criterion validities. Such data is expected to become available during the time of our present publication.

IV. ADMINISTRATION OF THE J.P.Q. AND CALCULATION OF VARIANCE VALUES

Our aim was to test a sufficient population of identical twins, fraternal twins, sibs together, sibs apart, unrelated pairs reared together and the general population—all in the 11–15 age range. What restrictions we could afford to keep, as to sex, social range, etc., depended on the relation between the population size we considered adequate, and the available population.

Prior to experiment there is no way of knowing what population sizes will give variances of adequate stability. We aimed ideally at 100 pairs in each category, and

reached this essentially in two categories: sibs together, and general population. But in spite of search in three major cities we finished, in the case of fraternal twins, with only some thirty-two pairs; in sibs apart, thirty-one pairs; and in unrelated children together, thirty-six pairs. Even in these short categories, however, we have about the same order of population size as the chief previous studies. (Newman, Freeman and Holzinger had 50 identicals and 50 fraternals. Eysenck and Prell had 25 identical pairs and 25 fraternal pairs.)

For the guidance of other researchers in this area it may be helpful to indicate that the number of cases obtained, even with the best school and press campaign, and the assistance of the principal social welfare and placement agencies falls short by perhaps 75% of the theoretically available cases in the area (calculated, for twins, for example, on the basis of one child in forty being born a twin). For this reason we found the Chicago area did not provide sufficient cases, and we were compelled to add Boston and New York. The *most* difficult cases to obtain were siblings reared apart from birth, and the next most difficult the unrelated children reared together.

Our deficiency of fraternal twins was due to our own error, of apparently asking in the press campaign for identical twins, whereupon we got a group which proved later to be largely identicals. More fraternals would otherwise have come in, and are coming in, in a supplementary study now in progress. As the data gathering proceeded it became evident that we could not get enough cases within the strictest form of homogeneity—same sex and male—so our goal was changed to same sex pairs, male *or* female. It is relatively unimportant whether ratios first be obtained for more or for less homogeneous families, providing we know which the obtained variance ratios apply to, and providing we do not employ the within-family identical twin variance term for families of *un*like sex pairs. (In other words, the *we* and *wh* terms in the above equations need a multiplication of primes if we take pairs of differing sex composition. The former is necessary because of the culture pattern difference of treatment of boys and girls and the latter because of the *X* chromosome.)

We wish to express our great indebtedness to a wide range of people who assisted in this location of cases—the most difficult part of the research. In particular we are glad to record our thanks to Dr. Kenneth Lund and his assistants in the Chicago Schools' Psychological Clinic; to Dr. S. Cook of New York University; to the school superintendents and principals in Chicago, Evanston, Winnetka, and Champaign, Illinois; the Catholic Home Bureau and the City Department of Welfare in Chicago; the Massachusetts Division of Child Guardianship; the Catholic Home Bureau in New York City and the Angel's Guardian Home in Brooklyn, New York. Especially we express our indebtedness to Dr. Benjamin Burack and the authorities of Roosevelt College for generously providing central research rooms for the Chicago testing and to Dr. Norton Kristy for organizing case work. The children to be tested were brought to a central clinic or testing center, and responded to the J.P.Q. questionnaire under the immediate supervision of the psychologist. This was done in a quiet session after the objective tests, physical measurements and photographs had been taken. The results were obtained as raw scores, by the keys provided with the handbook, and the following computational procedures ensued.

1. *Correction for Age.* In those factors—B, I, J, K and Q_3—in which a significant

age trend has been found, all scores were corrected, by the table in the handbook (13), to the 11 year level. Thus all statements, e.g., about inter-sibling variance, must be considered to be independent of trend differences due to age. The reader should be alerted that this possible source of contamination of variance has not been eliminated in some studies with which ours may need to be compared.

2. *Calculation of Variances.* Wherever the pattern was that of two cases per family the within variance was most readily calculated by summing the squares of the differences of the two pair members; while the basis for the between variances was obtained by summing the squares of the corresponding sums. (These sums of raw scores, in the latter case are then converted by the usual $\Sigma X^2 = \Sigma X^2 - (\Sigma X)^2/N$). Both of the variance values thus obtained need to be halved because they are calculated on a doubled scale. Indeed, the *between* variance total would need to be divided by four if one were not already needing doubled values to fit the "mean square" equations 7 through 11, as explained above.

A check on the within variance may be obtained by working out the intra-class correlation coefficient, as is commonly used in correlating twins.

The degrees of freedom for these variances are (1) $km - k$ for the within variances, m being the number in each family, i.e., 2, and k the number of families. (The d.f. are thus equal to the number of families) and (2) $k - 1$ for the between variances. In a few instances, in unrelated reared together, we had data for three in a family, and here it is necessary to calculate means throughout, allowing two degrees of freedom for the within variance in such a family.

3. *Correction for Experimental Error of Measurement.* As indicated in the above equations, the variance as obtained in (2) consists of true variance inflated by error variance, and our nature-nurture relations are required only for the estimated true factor variances. Since the error is $\sigma^2(1 - r)$ the true values on the left are computationally obtained by multiplying the obtained values by the consistency coefficient.

At this point two alternative procedures become possible. We can either take the best estimate of the consistency coefficient for the "population", from which twins, sibs, etc., are samples; or we can accept the value for each sample as an indication of the error in that sample. Some statisticians have recommended the former, but it must be recognized that we are in fact dealing with both sampling error and experimental error. The latter may indeed vary from sample to sample through other than sampling errors and this *should* be reflected in the correction. Retaining a constant population figure for consistency on one factor throughout all equations would not give a different solution from using uncorrected variances (for the left side of every equation would be reduced in exactly the same proportion), and the first alternative would therefore make sense only if the consistency coefficient were modified in each sample according to the variance, which is naturally systematically different for twins, sibs, etc. This is done by

$$\sigma_z(1 - r_z) = \sigma_p(1 - r_p)$$

where we know (by estimate) r_p, the consistency in the general population, σ_p for the population, and σ_z for the special sub-group.

In another analysis we shall recompute the solutions on the basis of variances uncorrected for error, to see how much difference results. But here we have followed what seems to us the best course, namely, to correct. And we have compromised between the two extremes of such correction, taking the mean of the r_z obtained as above and the consistency r obtained for the actual sample. For using the latter alone, empirically obtained on small samples, and for factors of lower reliability, produces gross variations in the estimated error-free variance, whereas the former cannot strictly be accepted because it ignores real differences in reliability of testing from sub-group to sub-group.

V. THE EXPERIMENTAL DATA AND ITS TREATMENT IN THE SIMULTANEOUS QUADRATIC SOLUTIONS

Table I(a) sets out the actually obtained variances as derived from raw scores (possible range 0–12) for each of the 12 factors in the J.P.Q., corrected to 11 years of age as indicated above. Table I(b) contains the corresponding consistency coefficients (reliabilities) calculated as the mean of the standard-deviation-corrected general population value and the value empirically obtained in the sub-group concerned.

As indicated earlier, a complete solution for eleven unknowns—six variances and five of the six correlations—by the eleven equations above was found to be impossible because five of the equations turn out to be linearly dependent upon the remainder. This arises largely from the supplementary relation of within family variances in the first five equations and between family in the last five, with respect to equation six.

TABLE I

(a) EXPERIMENTALLY OBTAINED, OBSERVED VARIANCES

Group	Factor											
	1	2	3	4	5	6	7	8	9	10	11	12
Identical Twins	2.27	3.05	2.55	2.51	2.70	2.21	2.43	3.47	4.30	2.21	1.89	1.40
Fraternal Twins	3.33	4.75	4.09	2.71	3.64	2.39	3.27	4.83	3.86	3.48	2.78	2.68
Sibs Together	3.44	4.66	3.57	3.52	2.79	2.40	2.99	4.78	3.87	3.24	2.65	3.18
Sibs Apart	4.09	6.16	4.29	4.21	2.77	3.80	4.16	5.38	4.88	3.29	3.00	3.29
Unrelated Together	3.53	5.72	4.05	3.88	2.94	3.96	6.42	4.63	5.29	5.81	4.05	3.41
General Population	5.04	5.55	4.00	4.83	3.72	3.31	5.87	4.65	5.49	3.47	4.10	3.73

(b) RELIABILITIES OF MEASUREMENT

(Mean of two estimates)

Group	Factor											
	1	2	3	4	5	6	7	8	9	10	11	12
Identical Twins	.60	.55	.47	.35	.27	.19	.30	.47	.31	.24	.44	.65
Fraternal Twins	.51	.59	.38	.40	.23	.18	.30	.45	.35	.22	.48	.58
Sibs Together	.68	.61	.44	.49	.30	.20	.37	.53	.38	.33	.56	.55
Sibs Apart	.54	.56	.47	.44	.26	.24	.48	.59	.44	.42	.40	.69
Unrelated Together	.68	.53	.42	.49	.31	.25	.26	.53	.38	.20	.37	.58
General Population	.67	.55	.52	.47	.25	.32	.31	.54	.37	.34	.41	.63

But even if we had not encountered this, some indeterminacy would remain because of the quadratics and the spreading ambiguity of equally possible solutions.

Accordingly, and since four of the six r's are almost certainly zero, we decided to take five equations and solve for five variances, fixing the two r values in ways to be explained. At this point we had a choice of which of two linearly dependent equations to drop, and we decided on the following bases:

(1) The relative importance of the unknowns involved. The variances, as indicated, were considered more important than r's.

(2) The size of samples on which we had data at the time of this decision. On this basis we decided to drop either sibs reared apart or fraternal twins as too few.

(3) The desirability of dropping equations involving r's that could not so confidently be considered zero. Actually $r_{we,wh}$ and $r_{be,bh}$ had to be retained for it is these alone that cannot be considered zero.

Although our choice was a careful one, it is still possible that a better combination of five equations than ours can be found, and certainly others exist. In this compromise we are forced to two assumptions we had wished to avoid, namely, (1) we assumed there are only two kinds of within family environmental variance—that of sibs and identical twins and that of fraternal twins. (2) we assumed only the correlations of *environment and heredity* to be significant, and only in the same realm (within family or between family). These are therefore *not* eliminated, as stated above. They can only be handled, however, either by ulterior empirical evidence as to what they should be, or as here, by assigning a reasonable range of values to them, which permits (a) seeing how much the variances are affected by such a range and (b) obtaining a *range* of variance solutions each conditional on an explicitly assumed correlation.

Although our results are reported on the second basis we have also explored the first, by taking examples where an appreciable hereditary variance exists and is tolerably known, and here we tended to find $r_{we,wh}$ with low negative values. This would agree with expectations from *the law of overt and covert trait deviation* (5), though this applies to traits where deviations are socially undesirable. It implies a general tendency of society, within and without the family, to bring environmental pressure to bear on *deviant* hereditary tendencies, i.e., to press all individuals toward the social norm presented by the existing mean for any trait. This is unlikely to apply to such desirable traits as intelligence, ego-strength and super ego-strength, and, indeed, our final results suggest there are more positive than negative (8 to 4) relations of heredity to environmental influences. However, *a priori*, either might arise, for even in intelligence it may be pointed out that society allows the bright to mark time while it concentrates special education on raising the less bright.[1] Our final results

[1] We find it widely assumed in the literature that the parable of the talents applies, and that the more intelligent get the more intelligence-provoking environment (see 1, 24, 31 and 46 in (8)). This, as we have argued elsewhere (4) is surely a naive and question-begging assumption. For example, both among school teachers and among the parents whom we questioned, even the consciously accepted "obvious thing to do" was more frequently to concentrate intellectual stimulation around the less bright. However, since the converse view was also met we have entered the intelligence equations, just like those for other factors, with both positive and negative correlations, in alternative solutions.

suggest culture tends to be negatively related to heredity where heredity is powerful, but otherwise positive relations predominate.

In general, through lack of space for specific discussion we have assumed within and between family heredity-environment relations have the same sign; though differences could sometimes be justified.

Although the available evidence thus suggests both positive and negative values according to social circumstances, the only acceptable procedure in handling this r, which cannot be assumed zero and yet which cannot be determined, would seem to be to take a spread of values through the most likely range and see how much the resulting variances are affected by this range of assumptions. Accordingly we have systematically, in all factors, tried four values: ± .10 and ± .50, while in intelligence we have also tried ± .30 and ±.60.

The equations finally used from Section 2 above were 1, 2, 3, 5, and 6—the equations for sibs raised apart and for the "between variances" being dropped. However, another solution, using all available data and equations, by the method of least squares, has also been made and will later be reported.

For purposes of easy computation the five equations used—1, 2, 3, 5 and 6 above—were solved for the standard deviations, whereby the latter could be calculated by putting in the empirical values directly. The solutions are:

(1) $\sigma_{we} = \sqrt{\sigma^2_{ITT'}}$

(2) $\sigma_{be} = -r_{be\cdot bh}\sqrt{\sigma^2_{UT'} - \sigma^2_{ST'}} \pm \sqrt{\sigma^2_{UA'} - r^2_{be,bh}\sigma^2_{ST'} - (1 - r^2_{be,bh})\sigma^2_{UT'}}$

(3) $\sigma_{wh} = -r_{we\cdot wh}\sqrt{\sigma^2_{ITT'}} \pm \sqrt{\sigma^2_{ST'} - (1 - r^2_{we\cdot wh})\sigma^2_{ITT'}}$

(4) $\sigma_{bh} = \sqrt{\sigma^2_{UT'} - \sigma^2_{ST'}}$

(5) $\sigma_{we''} = -r_{we\cdot wh} \pm \sqrt{\sigma^2_{rTT'} - \sigma^2_{wh}(1 - r^2_{we,wh})}$

For uniformity, all solutions are kept as standard deviations and need squaring to give variances. They are best solved in this order, since earlier values are needed to insert in later equations. These equations represent only the positive solutions, since a negative standard deviation is meaningless. Even so there are potentially two solutions for σ_{wh} and $\sigma_{we''}$, though in all cases it happens that one vanishes, being negative. The question of the particular correlation values to be inserted is taken up in the next section on particular factors. Where four different r's are tried there are of course, typically four solutions for σ_{wh}, σ_{be} and $\sigma_{we''}$, as shown for each factor below.

The actual empirical variances on which these equations are worked are those set out in Table II, representing corrected values, though, as stated, a separate study will also report the use of uncorrected variances. Since these variances (for any one factor, in different sib, twin, etc., samples) should not behave as samples from a single population we have applied the F test to a random 100 pairings and obtained

TABLE II.—CORRECTED, "TRUE" VARIANCES FROM THE FIVE KINDS OF GROUPS USED

Group	1	2	3	4	5	6	7	8	9	10	11	12
Id. Twins Together	1.36	1.68	1.21	.88	.73	.42	.73	1.63	1.33	.53	.83	.91
Frat. Twins Together	1.70	2.80	1.52	1.08	.84	.43	.98	2.17	1.35	.77	1.34	1.55
Sibs Together	2.34	2.84	1.57	1.73	.84	.48	1.11	2.27	1.47	1.07	1.48	1.75
Unrelated Together	2.40	3.03	1.70	1.90	.91	.99	1.67	2.45	2.01	1.16	1.50	1.98
General Population	3.38	3.05	2.08	2.27	.93	1.06	1.82	2.51	2.03	1.18	1.68	2.35

TABLE III.—SIZES OF SAMPLES AND DEGREES OF FREEDOM

Identical Twins	Fraternal Twins	Sibs Together	(Sibs Apart)[1]	Unrelated Together	Unrelated Apart[2]
104 d.f. = 52	64 d.f. = 32	182 d.f. = 91	(62) (Not used)	72 d.f. = 36	540 d.f. = 539

[1] Not used in this analysis.

[2] General Population. In this case d.f. = 539 since they are not taken in pairs but from a common mean.

69% of differences significant at the 1% level and 26% at the 5% level, while 5% did not break the null hypothesis.

The general population variance used here (originally in Table I) is the mean of two estimates: (1) from taking one child from each pair, i.e., a random representative from each of our 210 families, and (2) from a second and larger sample of 330, 11–15 year old boys and girls from towns in Illinois. The general population variance is thus understood, by our age correction of scores, to be the variance of a *one year (11 year old) cross section of the population*, including no two members of the same family (except for the one case in forty which is a twin). The numbers in the other groups, and resulting degrees of freedom, are shown in Table III.

VI. THE CONTRIBUTORY VARIANCE RATIOS DISCOVERED FOR PERSONALITY FACTORS

It is proposed now to set out the obtained variances and ratios, factor by factor. These give four values (set out in corresponding order) for σ_{be} and σ_{bh} (theoretically eight, but four are always negative), and possibly up to eight for $\sigma_{we''}$, but actually never more than five.

Although, in general, the alternative r's rarely carry the resulting ratio from a predominantly hereditary to a predominantly environmental one, or vice versa, it is desirable to indicate in each case an r value preferable on some grounds to the others, and to accept consistently the other values which go with this. In this, our considerations, apart from specific ones, brought in below, are: (1) Decided correlations (say .50 or more) between personality and sociological influences are rare. Other things being equal, we should prefer the 0 or $\pm$ .10 values. (2) A negative or positive r is to be preferred according to whether or not the trait is one which society seeks to hold in check at its extremes. (3) The r associated with the more central value among the alternatives is to be preferred, rather than a more extreme estimate.

(4) In general we may expect rather smaller relations of heredity and environment within society ($r_{be.bh}$) than in the family, ($r_{we.wh}$), since the person's qualities are more constantly understood in the family. However, in one case, intelligence, we know of some appreciable $r_{be.bh}$ values, notably of +0.3 between intelligence and social status (4)(35). (5) Most guidance can perhaps be gained from the assumption that the relation of σ^2_{bh} and σ^2_{wh} is likely to stay in fairly narrow limits. This is because we are dealing with a relation common to most hereditary mechanisms and little affected by specific social conditions. In many cases this at once rules out all but one of the four alternative algebraic solutions for σ_{wh}, and at the same time indicates the preferred $r_{we.wh}$ correlation. Two possibilities[1] have to be considered in this last, but they indicate ratios of σ^2_{bh} to σ^2_{wh} probably only ranging from 2 to 1 to 1 to 2.

Factor I. Commonly called "I": Tender-minded vs. Tough-minded
(Also Sensitive, Anxious, Emotionality vs. Tough Poise)

Computed values:

σ^2_{we} = *1.36* ($\sigma^2_{we'}$ = *.77* or .64 (r = ± 10); *.86* (r = + 50)

σ^2_{wh} = *.77* and 1.23 (r = ± 10); *.32* and 2.99 (r = ± 50)

σ^2_{be} = *.93* and 1.03 (r = ± 10); *.74* and 1.26 (r = ± 50)

σ^2_{bh} = *.06*

The above form will be followed in setting out all factor values. The four values correspond to the four possible correlations, in the order indicated. A total consistency will decide the preferred solution, and in this the *wh/bh* ratio will be given prime consideration, but also the psychologically preferred r and the central tendency of the estimates, as well as the closeness of *we'* to *we*. In this case the + 50 (or, less well, the +10) gives the best agreement of *we* and *we'* (choice of latter underlined) and also gives the only value, .32, for *wh* that brings it nearest the expected order of relation to *bh*. Accepting this system we find that between families, environmental differences are of the order of twelve to fifteen times as effective as hereditary differences in accounting for individual differences in *I*. Correspondingly, within the

[1] They are: (1) with random, unassortative mating and (2) with some degree of positive correlation of husband and wife. In the latter the between family hereditary variance is likely to climb relative to within variance. Parenthetically, no such guidance can be sought from an inherent law of relationship of "within" and "between" *environmental* variance. For the relationships there are not beyond the control of man and are subject to all sorts of specific and culturally fluctuant manipulations. But the hereditary ratio of σ^2_{wh} and σ^2_{bh} should be constant within limits useful enough to guide our choice among the four mathematically alternative answers for σ^2_{wh}, (for, in our calculations at least, σ^2_{bh} is free of ambiguity). For an estimate of this ratio we can turn (a) to general evidence from other genetic fields, which points to something of order of 1:2 in non-assortative mating for the ratio of σ^2_{wh} to σ^2_{bh}, and (b) to a calculation from the variance of the mid-parent relative to the total society, which as pointed out below, would make σ^2_{bh} greater than σ^2_{wh}. The mean value for all our factors on all estimates, of σ^2_{wh} and σ^2_{bh}, actually gives a ratio of 3.6 to 1. Since there is no reason to suppose the hereditary mechanism is other than polygenic, for all these personality dimensions the preferred σ^2_{wh} value should be the one approximating a ratio of between 1 to 1 and 1 to 2 to the fixed σ^2_{bh}.

family, environment predominates, being about four (two to five) times as important as heredity. The values finally chosen are italicized.

In short this pattern is largely environmentally determined, and on more accurate analysis might prove to be wholly an environmental mold trait (6). The larger ratio for *between* family environment suggests that this resides in some sort of family atmosphere—almost certainly an over-protective, gentler tradition as opposed to a Spartan roughness. The size of positive correlation suggests some selection of gentler temperaments to the gentler environment.

Factor 2. Commonly called Q_4. Nervous Tension vs. Autonomic Relaxation (Also called "Somatic Anxiety" (12))

σ^2_{we} = *1.69* ($\sigma^2_{we''}$ = 1.64 ($r = \pm 10$); *1.64* ($r = +50$) 1.52 and .52 ($r = -.50$)

σ^2_{wh} = .92 and 1.49 ($r = \pm 10$); *.37* and 3.63 ($r = \pm 50$)

σ^2_{be} = .01 and *.03* ($r = \pm 10$); *.01* and .23 ($r = \pm 50$)

σ^2_{bh} = *.19*

Again the correlation which makes for most consistency is one of about +50. In such consistency, it should be pointed out, we do not assume that the ratio of *bh/be* should equal that of *wh/we*, nor that the correlation should be the same in the two cases. However, one might reasonably expect them to be in the same direction, in general, and of the same sign, in the case of r, with $r_{be,bh}$ lower.

The bulk of the variance in this factor is within families. Taking the σ^2_{bh} value, .37, which alone stands in any acceptable ratio to σ^2_{bh}, we find environment predominating, standing four times as influential as heredity *within* families, and about twice as important *between*.

This is perhaps not unexpected, clinically, in that we are dealing with one of the major forms of neurotic anxiety. Presumably the intra-family events are those early experiences so emphasized by psychoanalysts and others. Incidentally, in general, it is probable that large emphasis on *within family* environment generally means emphasis on early life effects, since the effects of the family status within society—the interactions of family with society—are not usually operative until later.

Factor 3. Commonly called "C" (or "O"): General Neuroticism vs. Ego Strength

σ^2_{we} = *1.21* ($\sigma^2_{we''}$ = 1.21 and 1.21 ($r = \pm 10$); *1.21* ($r = +50$), 1.23 and .05 ($r = -50$)

σ^2_{wh} = .25 and .52 ($r = \pm .10$): *.06* and 1.86 ($r = \pm .50$)

σ^2_{be} = .34 and .40 ($r = \pm .10$); *.20* and .69 ($r = \pm 50$)

σ^2_{bh} = *.13*

In this case the most central tendencies and those best in keeping with the within family hereditary variation being of the same order as the "between," are those de-

rived from an r between hereditary proneness and environmental provocation of about +.40.

Whatever r is adopted, the predominant influence is to environment, in within and between family situations together. The preferred r would give a 10 to 1 relation within the family and about 2 to 1 as between families.

This result, to our surprise, favors the standard clinical and psychoanalytic viewpoint and possibly the degree of hereditary determination found by Scott in animals (29) rather than the recent major emphasis on heredity in neurosis by Eysenck and Prell (18). Within environment the weight of causation is partly on the total family atmosphere but more on the specific happenings to individuals within the family. Briefly, the following reconciliations may be suggested. (1) We believe Eysenck and Prell's factor of neuroticism is different from our C, being based on (a) less complete factor extraction and (b) on determination by criterion rotation rather than simple structure. Criterion rotation could lump together parts of several distinct factors distinguishing neurotics from normals. Our Q_4, for example, is probably included in such a composite. (2) Eysenck's samples were smaller and, as has been usual in twin studies, no correction was made for difference between the test measurement and the factor measurement. (3) Correlation of variances was not allowed for in that study. Accordingly, we feel entitled to conclude that the greater environmental variance found here is likely to be nearer the truth.

Factor 4. Commonly called Q_3: Will Control (13)

σ^2_{we} = *.88* ($\sigma^2_{we''}$ = *.29* and .10 ($r = \pm$ 10), *.40* ($r = +$ 50))

σ^2_{wh} = *.13* and .30 ($r = \pm$ 10); *.03* and 1.25 ($r = \pm$ .50)

σ^2_{be} = *.33* and .42 ($r = \pm$ 10); *.18* and .72 ($r = \pm$ 50)

σ^2_{bh} = .17

A moderate positive correlation, about .20, of within environment and within heredity, gives the most consistent relation here. This, or even fairly wide variations from it, will give environment a predominance over heredity reaching about 8 to 1 within the family. Although superficially the pattern of Q_3 looks like one of acquired precision, moral standards and self discipline, it must evidently be founded appreciably also on an hereditary pre-disposition which decides how far this self concept will "take" as between one family and another. A rather unusual discrepancy of twin and sib environmental variance here requires psychological explanation.

Factor 5. Commonly called "D": Impatient Dominance, Immaturity or Sthenic Emotionality (6) (15)

σ^2_{we} = *.73* ($\sigma^2_{we''}$ = .72 ($r \pm$ 10) .72 ($r = +$ 50) .72 and .02 ($r = -$50))

σ^2_{wh} = *.07* or *.19* ($r = \pm$ 10), .01 or .95 ($r = \pm$ 50)

σ^2_{be} = *.01* or *.03* ($r = \pm$ 10); .004 or .11 ($r = \pm$ 50)

σ^2_{bh} = *.07*

A zero or slightly negative r is all that is indicated here. Practically all solutions make most of the variance in this factor depend upon within-family environment.

This finding agrees with some as yet unpublished evidence, that the level of the Impatient Dominance factor is related to degree of spoiling and favoritism encountered. It suggests that some particular family position influences could profitably be investigated as major determiners of this pattern.

Factor 6. Commonly called "A": Cyclothymia vs. Schizothymia (6) (13)

σ^2_{we} = *.42* ($\sigma^2_{we''}$ = .37 (r = ± 10), .37 (r = +50), *.29* or .04 (r = −50)

σ^2_{wh} = .04 and .10. (r = ±10); .01 and *.53* (r = ±50)

σ^2_{be} = .04 and .12 (r = ± 10); 01 and *.66* (r = ± 50)

σ^2_{bh} = *.51*

These figures suggest that mating may be less assortative on this trait than others (perhaps the non-easy-going seeks the easy-going) and/or that environment acts *against* hereditary deviations, with a negative r of perhaps −.50. For the first time we encounter a factor in which heredity is *more* important than environment within the family, and about equally important between families.

The higher emphasis on genetic influences here agrees with the findings of Kretschmer (21), Sheldon (30) and Stockard (32) of strong body build associations with cyclothymia vs. schizothyme tenseness, and the psychiatric findings of Kallmann (20), Rosanoff (27), Slater (31) and others, of appreciable hereditary determination of whether mental disorder shall take manic-depressive or schizophrenic forms. The environment of twins is indicated to be more alike than that of sibs in influence on this factor.

Factor 7. Commonly symbolized "H": Adventurous Cyclothymia vs. Withdrawn Schizothymia

σ^2_{we} = *73* (σ^2_{we} = .62 and .58 (r = ± 10), .62 (r = + .50))

σ^2_{wh} = .28 and .51 (r = ± .10), .10 and *1.39* (r = ± .50)

σ^2_{be} = .10 and .22 (r = ± .10), .03 and *.83* (r = ± 50)

σ^2_{bh} = *.56*

In this case an r of about −.50 to −.60 would seem to give the best fit and to agree also with psychological expectations. For education, both within and between families, aims to "bring out" the shy and to repress the unduly boisterous. Nevertheless the strongest hereditary determination yet encountered is found here, showing roughly equal action between families and a two to one predominance regarding within family variations.

The theory propounded elsewhere (6), that *A* and *H* represent hereditary and environmental factors in the schizothyme pattern must be modified, for unless later research alters the values *both* components have substantial hereditary roots.

Factor 8. Commonly called "K": Socialized Morale vs. Boorishness (6) (13) (14) *(Also called Trained Mind vs. Rejection of Education)*

σ^2_{we} = *1.63* ($\sigma^2_{we''}$ = *1.54* and *1.52* (r = ± .10), 1.54 (r = + 50), 1.31 or .27 (r = −50)

σ^2_{wh} = *.46* and *.88* (r = ± 10); *.15* and 2.76 (r = ± .50)

σ^2_{be} = *.04* and *.09* (r = ± 10); .01 and .29 (r = ± 50)

σ^2_{bh} = *.18*

Absence of correlation between the two influences gives the best internal consistency. Hereditary influence then predominates more than two to one between families, while the reverse holds within families. By the apparent psychological nature of the factor, a larger environmental influence might be expected, though an interpretation as "temperamental susceptibility" to culture has also been previously hypothesized (6).

Factor 9. Commonly symbolized by "E": Dominance or Independence vs. Submissiveness

σ^2_{we} = *1.33* ($\sigma^2_{we''}$ = 1.21 and *1.20* (r = ± 10), *1.21* (r = + 50), *1.04* or .08 (r = −50))

σ^2_{wh} = .08 and *.26* (r = ± .10), .01 and 1.60 (r = ± .50)

σ^2_{be} = .01 and *.06* (r = ± .10), .001 and .58 (r = ± .50)

σ^2_{bh} = *.54*

One would almost certainly have to accept a negative correlation here, from the psychological evidence that our culture encourages the meek and frustrates the dominant; and indeed the −.10 correlations gives the best *wh/bh* relationship. Heredity would then be about ten times as important as environment *between* families, but one fourth as important within families. The latter suggests that the juxtaposition of highly dominant persons in one family results inevitably in some modification of dominance, to a greater extent than in the outside world.

These conclusions—hereditary emphasis and within family modification—fit the animal experimentation results (23) as well as, say, Sheldon's concept of mesomorphy, and the endocrine evidence.

Factor 10. Commonly called "J": Energetic Conformity vs. Quiet Eccentricity (6) (15)

σ^2_{we} = *.53* ($\sigma^2_{we''}$ = .25 and .18 (r = ± .10), *.30* (r = + .50)

σ^2_{wh} = .44 and .66 (r = ± 10), *.21* and 1.40 (r = ± .50)

σ^2_{be} = .01 and .03 (r = ± .10), *.003* and .12 (r = ± .50)

σ^2_{bh} = *.09*

The emphatic feature here is the predominance of within family variance. An r of about +.40 is probably indicated, and this would give a roughly 10 to 1 predominance to heredity between family and an environmental predominance within family of about 2:1. The large within variance and relatively small twin environment variance suggests a possible family position effect and a lack of relation to cultural and status variables.

Factor 11. Symbolized "F": Surgency vs. Desurgency (6) (13) (14)

σ^2_{we} = *.83* ($\sigma^2_{we''}$ = .70 or .69 (r = ± .10), *.72* (r = + .50)

σ^2_{wh} = .52 and 81 (r = ± 10), *.22* and 1.90 (r = ± .50)

σ^2_{be} = *.17* and .19 (r = ± .10); *.13* and 24 (r = ± .50)

σ^2_{bh} = *.02*

Small *bh* values are, by the nature of the equations, unstable, and one suspects this might be nearer, say, 0.6, corresponding more to the lowest *wh*. The interesting fact to those who have studied the surgency factor in various contexts is the predominance of environmental determination. "Between" families environment would be three to ten times as influential, and, if the above positive r is accepted, it could possibly reach four times the intra-familiar variance (though twice may be more likely). Family climate is evidently very important, as one would expect with a dimension which has been hypothesized to be general inhibition level.

This environmental emphasis should help in the conceptual distinction from the *H* factor, with which it is often phenotypically confused.

Factor 12. Symbolized by "B" (or "g"): General Intelligence (6) (13)

σ^2_{we} = *.91* ($\sigma^2_{we''}$ = .74 and *.67* (r = ± .10), .76 (r = + .50))

σ^2_{wh} = .68 and *1.03* (r = ± .10), .31 and 2.27 (r = ± .50)

σ^2_{be} = .32 and .43 (r = ± 10), *.17* and .79 (r = ± .50)

σ^2_{bh} = *.23*

Since correlation of husbands and wives on intelligence is of the order +0.40 to +0.50, the ratio of *bh* to *wh* can be assumed to be on the high side. This would, in spite of our theory of intelligence and environmental stimulation, compel us to accept a positive r, at least within the family, if not in school. Accepting an r of +.50 would make heredity more important than environment between families but less important within families. A negative r might even make sense *within* the family, and one of −.10 is tentatively taken, at which heredity predominates.

This substantial environmental influence is at first very surprising, for despite lapse of years, there is no reason to doubt the massive evidence summarized by such investigators as Schwesinger (28), Thorndike (33) and Woodworth (35) for a decidedly higher hereditary contribution than this. The present result might therefore

reflect either a systematic bias in the new method or a peculiarity of the intelligence test. If we consider the way in which the method has dealt with the whole range of factors we see that it has made some largely hereditary and others largely environmental, though there *does* seem to be some systematic tendency for the environmental ratios to run higher; but there is no independent evidence that this is definitely wrong.

Accordingly we do well to examine the test, which is typical of verbal intelligence tests. The senior author has proposed elsewhere that nature-nurture ratios should not be determined on verbal tests, which can be demonstrated to have considerable cultural association, but on Culture-Free, Perceptual tests (7). It happened that for the general purposes for which the J.P.Q. is intended (15) the verbal form was alone appropriate, but in the experiment reported later it was supplemented by the Culture Free Tests. The use of the same method on the new data may help decide whether our present assumption is correct; that the anomalous intelligence factor findings here are due to rather heavy educational content in the test itself.

SUMMARY OF CONCLUSIONS

1. A multiple variance analysis method (8) has been applied for the first time to psychological, heredity-environment analysis, attempting from five linearly independent equations (survivors of eleven possible equations) to determine five variances. The sample consisted of 104 identical twins, 64 fraternal twins, 182 siblings reared together, 72 unrelated children reared together and 540 children in the general population. The measures were for twelve personality factors (including intelligence as one) on the Junior Personality Questionnaire Test.

2. A critical evaluation of the findings requires more extended discussion of the confidence limits of the variance ratios than is possible here. Debate ranges overestimates that the 2½% limits of a 1 to 10 ratio are approximately 1 to 8 and 1 to 12 to estimates which give a standard error as large as the ratios obtained, in certain cases. The matter will be handled systematically in the pending publication applying exactly the same equations to objective test measures on the same samples.

Meanwhile, this presentation may be taken as a demonstration of the method and as providing estimates, admittedly not sufficiently reliable to stand by themselves, but capable of giving cumulative evidence when compared with future estimates by ourselves and others, as to the true values. Both this unreliability (as shown in the instability of some values such as σ^2_{bh}, which arise as fine differences between two observed variances), and the failure of some sample differences to refute the null hypothesis (section V above) now show that for good solutions the experiment must be carried out on a scale *at least two or three times as large* as ours. And, as our experience shows, this will require thorough case research in three or four large cities, and a generous endowment.

3. In each factor we have presented not so much a solution as a series of solutions, depending on various assumptions of correlation. In some cases variation of r proves to affect the solution relatively little, but in a few it would invert the relative importance of heredity and environment. To lessen the range we have attempted to

choose a preferred value on the basis of an expected $\sigma^2_{wh}/\sigma^2_{bh}$ ratio of roughly unity[1]; on psychological considerations; and on internal consistency of the results (predominance, in one factor, of heredity or environment, in both within and between relations). More attention could advantageously be given to determining the *wh/bh* ratio, from known highly inherited traits and in relation to known assortativeness of mating, for it frequently permitted an instant rejection of one of the superfluous algebraic solutions. Frequently, alternative algebraic solutions are negative or absurd, so that in fact the method gives more unambiguous answers than might be theoretically expected. It is proposed to check elsewhere on the present solutions by using a least squares method that will permit more of our equations to be used. A solution is also being based on variances uncorrected for test unreliability, to compare with the present.

4. In spite of uncertainties on individual values, there is in general considerable internal consistency, and consistency with psychological understanding of the nature of the factor.

Some factors are predominantly environmentally determined, consistently in both "between" and "within" estimates. These are I, Tender-Mindedness; C, General Neuroticism; F, Surgency-Desurgency; Q_3 Will Control, and Q_4, Somatic Anxiety. However, in the neuroticism and anxiety factors heredity has an appreciable role as *between* families.

Four factors show about an equal role of heredity and environment but with heredity predominating *between* families; *J*, Energetic Conformity, *E*, Dominance, *K*, Socialized Morale, and *D*, Impatient Dominance.

Some three factors have larger roles for heredity than environment. These are: *A*, Cyclothymia vs. Schizothymia; *H*, Adventurous Cyclothymia vs. Submissiveness, and, *B* or General Intelligence.

The inferred correlations of hereditary and environmental influences range from $-.60$ to $+.50$, but most are positive. The strong negatives occur with *A*, *H* and *E*, which could be considered socially undesirable at either extreme, but the same might be said of *F*, where the indicators were of a positive *r*. In general the negative correlations are found with more highly inherited traits.

Some observations of interest to psychologists have been made at appropriate points regarding the relation of these conclusions to other psychological evidence, e.g. the twin studies on neuroticism and intelligence, body-build data, animal experiment. In the main there is convergence, but the present findings give a somewhat larger role to environment, and particularly to within family environment, in neuroticism, anxiety and verbal ability than do the twin studies. A number of provocative indications arise for psychological research *per se*, in terms of the direction to look for origins of particular source trait patterns e.g. family position.

The writers wish to thank those who have given help in the statistical discussion (but who are not responsible for our particular conclusions). In addition to our specific indebtedness to Dr. C. R. Rao, mentioned above, we had invaluable help

[1] The actual ratio of the mean *wh* variance to the mean *bh* variance accepted for these twelve factors is 1.6. With random mating and simple averaging of parental values, one would expect the *bh* value to be larger than this implies. A problem remains to be solved here.

from Dr. W. G. Madow and Dr. C. R. Blythe of the University of Illinois, Dr. D. R. Saunders of the Educational Test Service, and from an unknown member of the editorial board of this journal.

This research was supported in major part by a research grant from the Institutes of Mental Health, of the National Institutes of Health, United States Public Health Service.

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Some Problems in Maternal Age

ROBERT S. KROOTH

THE PROBABILITY that a human being will display certain characters seems to depend in part upon factors associated with the age of his mother at the time he is born. Generally, a birth is an event which can be described in terms of five variables:

1. The age of the mother ('maternal age').
2. The age of the father ('paternal age').
3. The total size of the sibship immediately after the birth of the newly born ('birth rank', 'order of birth' or 'parity' of the newly born).*
4. The fertility of the mating which produces the newly born (usually measured as the total, i.e. the *final* size of the sibship of which the new born is a member.)
5. The time-rate at which the parents reproduce (often expressed as the interval between the mother's pregnancies, particularly the intervals which just precede and just follow the birth of an affected).

Each of these variables is positively correlated with the variable which immediately follows it. The older a woman is at a particular time, the older her husband is likely to be. The older a pair of parents are, the higher the birth rank of any child they produce is going to be. The higher a child's birth rank, the more fertile his parents will be, and, generally, the more fertile a pair of parents are, the more rapidly will they seem to reproduce.

When the probability of a child displaying a certain character depends upon the age of his mother at the time of his birth, the phenomenon is spoken of as a maternal age effect. In the broadest sense the study of maternal age effects involves firstly, the measurement and description of the influence of a single one of the five variables listed above, and secondly, the separation of the effects of one variable from the effects of other variables with which it may be correlated. The literature on maternal age effects has been reviewed by Thurstone and Jenkins (1933) and Krooth (1952), the latter author being concerned primarily with the role of maternal age in the aetiology of congenital abnormalities. In this paper, attention will mainly be directed to the effects of maternal age alone, but the fact that maternal age is only one of a set of intercorrelated variables means that other avenues of analysis, complementary to the ones presented here, are also necessary.

Once it has been established that, with respect to some character, the maternal ages of affected children differ significantly from the maternal ages of 'normal' or unaffected children, two questions may be asked: First, what is the *law* which governs the way probability of affection (or 'risk') changes with maternal age? Second, what is the *importance* of maternal age in the causation of the character? This same ques-

Received September 14, 1954.

* There is some slight variation between countries (and between authors) with regard to the exact definition of birth rank.

tion, asked somewhat differently, would be: What is the *strength of association* between risk and maternal age?

Methods for answering these questions will now be considered.

1. THE 'LAW' OF A MATERNAL AGE EFFECT

The simplest way to find the law of a maternal age effect is to plot (or tabulate) the incidence of the condition at each maternal age against maternal age itself (Yerushalmy 1945, Carter and McCarthy 1951, etc.). This, however, is not always feasible for two reasons:

1. In small samples random fluctuations in the number of affected children at each maternal age may serve partially to conceal or distort the regression of incidence on maternal age.

2. It is only rarely possible to obtain a population, comprised of both affected and unaffected children, which allows the direct evaluation of the incidence of the character at each maternal age. More often the investigator is confronted by a sample of affected children and an external control group of some sort.

To overcome these difficulties special methods have to be devised.

A. Algebraic approach

The problem can be simplified by assuming that the distribution of affected children with respect to maternal age and the control population distribution of maternal ages are both approximately Gaussian. Now suppose that Y_2 is the proportion of affected children born at maternal age x, and that Y_1 is the proportion of unaffected children who are born at maternal age x, and let the ratio of affected children to unaffected children in the population be as $N_2:N_1$. Then the probability that a child born at maternal age x will be affected is $\frac{N_2Y_2}{N_2Y_2 + N_1Y_1}$. If $Y_2 = (2\pi V_2)^{-1/2} \exp\left[\frac{(x - m_2)^2}{2V_2}\right]$ and $Y_1 = (2\pi V_1)^{-1/2} \exp\left[\frac{(x - m_1)^2}{2V_1}\right]$ then the natural logarithm of the ratio of affected to unaffected at maternal age x is

$$\log\left(\frac{N_2Y_2}{N_1Y_1}\right) = x^2\left(\frac{V_2 - V_1}{2V_1V_2}\right) + x\left(\frac{m_2V_1 - m_1V_2}{V_1V_2}\right) + \frac{V_2m_1^2 - V_1m_2^2}{2V_1V_2} + \tfrac{1}{2}\log\left(\frac{V_1}{V_2}\right) + \log\left(\frac{N_2}{N_1}\right)$$

or, since the total incidence of the character may not be accurately known, the equation

$$Y = x^2\left(\frac{V_2 - V_1}{2V_1V_2}\right) + x\left(\frac{m_2V_1 - m_1V_2}{V_1V_2}\right) + \frac{V_2m_1^2 - V_1m_2^2}{2V_1V_2} + \tfrac{1}{2}\log\left(\frac{V_1}{V_2}\right)$$

will be referred to as a conditional equation. A constant must be added to Y to obtain the logarithm of the odds on affection.

Now if the abnormality is fairly rare over the effective range of maternal ages, $\frac{N_2Y_2}{N_2Y_2 + N_1Y_1}$ will not differ much from $\frac{N_2Y_2}{N_1Y_1}$, the odds on affection. Since this con-

dition of rareness is satisfied by all the abnormalities which will subsequently be considered, the odds on affection at maternal age x will be treated as an (approximate) estimate of the incidence of the character at maternal age x, and, by the same token, it will be assumed that the use of a national birth population as a control, rather than a population composed exclusively of unaffected, is sufficiently accurate. Y may thus be regarded as approximately transformable, by the addition of a constant, to the logarithm of the incidence of the abnormality at each maternal age.

It will be seen that when the variances of the control distribution and the affected distribution differ the conditional equation describes a parabola on a graph of Y against maternal age. When the variances are equal, the conditional equation is linear. It is of interest to define

$$T = \tan\theta = \frac{dY}{dx} = \frac{(V_2 - V_1)x + m_2V_1 - m_1V_2}{V_1V_2}$$

where θ is the angle the tangential line makes with the abscissa. When the conditional equation is at a maximum or minimum $T = 0$, and $x = \dfrac{m_1V_2 - m_2V_1}{V_2 - V_1}$, a result of use in discriminatory analysis (Penrose 1947).

Two interesting values of T occur when $x = m_1$ so that $T = \dfrac{m_2 - m_1}{V_2}$ and when $x = m_2$, so that $T = \dfrac{m_2 - m_1}{V_1}$.

Clearly, it is easier to manipulate the natural logarithm of the risk (or of the odds on affection) than it is to manipulate the probability itself. The tangents to the locus of the conditional equation at various critical points enable one to describe the system more fully. Note that the conditional equations for a set of independent conditions are additive.

The considerations discussed above are very similar to those employed in statistical discrimination (Smith 1947, Karn and Penrose 1951, Krooth 1953).

B. Empirical approach

This method of analysis was originated by Jenkins (1933). Suppose that M_x is the number of affected children born at the maternal age x, and that N_x is the number of children expected on the basis of the proportion of children in the general population born at maternal age x. Then define $R_x = \log \dfrac{M_x}{N_x}$. R_x is directly comparable with Y, or, more accurately, with $\log \dfrac{N_2Y_2}{N_2Y_2 + N_1Y_1}$ which we approximate by Y, and can be converted to the natural logarithm of the incidence of the character at age x simply by adding to it the logarithm of the total frequency of the character in the general population.

Now N_x is a number which in maternal age work is usually based upon so many individuals that it may be regarded as a constant. Then $V_{M_x} = M_x$, assuming the character is fairly rare; $V_{R_x} = \left(\dfrac{\partial R_x}{\partial M_x}\right)^2 (V_{M_x}) = M_x^{-1}$. Hence I_{R_x}, the quantity which

gives a measure of the information pertaining to R_x, is M_x, the observed number of affected at the maternal age x.

C. Analysis of Data

The methods of analysis described above have been applied to four separate conditions: mongolism, congenital heart disease, achondroplasia and twinning. The material used is summarized in Table 1. Each series of cases has been compared with a national birth population, which has been selected so as to conform, as nearly as possible, with the series itself. In all of these data the difference between the corresponding observed and control distribution is statistically significant.

The twin series originally came from the records of all multiple births in England and Wales during 1939, the number of twins of each type being determined by Weinberg's Differential Method (McArthur 1949). With the exception of the Galton Laboratory series, none of the mongol data contains (known) familial cases of mongolism, i.e. cases of mongolism where a relative was also affected.

Table 2 gives the conditional and tangential equations. The parameters describing the respective maternal age distributions were estimated from grouped data, the frequencies being given for the maternal age intervals 15–19, 20–24, etc. In order that the midpoint of each group might be integral, the origin of measurement was taken at 0.5 years. The variances were reduced by Sheppard's correction for grouping. Figures for the control populations were obtained from the 1950 Demographic Yearbook of the United Nations.

Some of the more characteristic properties of the parabolas are given in Table 3.

It will be noticed that the probability of mongolism seems to increase the most rapidly with maternal age judging by the tangents at the two means. The tendency for dizygotic twinning ranks next, although the effect is substantially less than that observed for the mongols. Congenital heart disease and monozygotic twinning show only a slight effect, the former being the more pronounced of the two. Achondroplasia

TABLE 1

Character	Original Author	Source	Number of Cases	Control Birth Population*
Mongolism	Penrose	Penrose (1951)	260	Eng. & Wales 1939
	Van der Scheer	Penrose (1951)	316	Netherlands 1936
	Orel	Penrose (1951)	104	Germany 1937
	Beall & Stanton	Penrose (1951)	121	Canada 1936
	Hanhart	Penrose (1951)	52	Switzerland 1936
	not fully published	Galton Laboratory	199	Eng. & Wales 1939
Achondroplasia	not published	Galton Laboratory	19	Eng. & Wales 1939
Congenital heart disease	not published	Galton Laboratory	193	Eng. & Wales 1939
Dizygotic twinning	R.-G. Eng. & Wales	McArthur (1949)	4974	Eng. & Wales 1939
Monozygotic twinning	R.-G. Eng. & Wales	McArthur (1949)	2185	Eng. & Wales 1939

* Information concerning the 1939 population of England and Wales was obtained from the Registrar-General's (1944) *Statistical Review*. The other control data came from the Demographic Yearbook of the United Nations.

TABLE 2

Character	Conditional Equation	Tangential Equation (dy/dx)
Mongolism:		
1. Penrose	$Y = 0.00433x^2 - 0.0724x - 2.39$	$T = 0.00865x - 0.0724$
2. Van der Scheer	$Y = 0.00304x^2 - 0.0243x - 2.65$	$T = 0.00609x - 0.0253$
3. Orel	$Y = 0.00604x^2 - 0.241x + 1.33$	$T = 0.012x - 0.241$
4. Beall & Stanton	$Y = 0.00364x^2 - 0.0841x + 1.61$	$T = 0.00728x - 0.0841$
5. Hanhart*	$Y = 0.000509x^2 + 0.176x - 6.38$	$T = 0.00102x + 0.176$
6. Galton Laboratory*	$Y = 0.000985x^2 + 0.106x - 6.26$	$T = 0.00197x + 0.160$
Congenital heart disease†	$Y = 0.00542x^2 - 0.270x + 3.16$	$T = 0.0108x - 0.270$
Achondroplasia	$Y = 0.154x - 4.79$ (Linear)	$T = 0.154; \theta = 8.75°$
Dizygotic twinning	$Y = -0.00201x^2 + 0.168x - 3.08$	$T = -0.00402x + 0.168$
Monozygotic twinning	$Y = 0.00109x^2 - 0.0395x + 0.152$	$T = 0.00218x - 0.0395$

* In these two series of mongols, the variances were not significantly different, and linear equations have therefore been fitted:—

Source	Conditional equation	Tangent
Hanhart	$Y = 0.212x - 6.99$	$T = 0.212; \theta = 12.0°$
Galton Laboratory	$Y = 0.232x - 7.55$	$T = 0.232; \theta = 13.1°$

† Corrected for an independent parity effect.

TABLE 3

Character	T_{x-m_1}	θ_{x-m_1}	T_{x-m_2}	θ_{x-m_2}	Coordinates of Vertex	Length of Latus Rectum*
Mongolism						
Penrose	0.175	9.9°	0.249	14.0°	8.4 yrs., − 0.70	924 yrs.
Van der Scheer	0.156	8.9°	0.200	11.3°	4.1 yrs., − 0.43	1310 yrs.
Orel	0.110	6.3°	0.181	10.3°	19.9 yrs., − 2.49	668 yrs.
Beall and Stanton	0.122	6.9°	0.175	9.9°	11.5 yrs., − 0.76	1100 yrs.
Hanhart	0.206	11.7°	0.212	12.0°	−173 yrs., − 15.7	7840 yrs.
Galton Laboratory	0.216	12.2°	0.232	13.1°	−81.2 yrs., − 7.21	4080 yrs.
Congenital heart disease	0.027	1.5°	0.036	2.1°	25.0 yrs., − 3.84	792 yrs.
Dizygotic twinning	0.023	3.1°	0.047	2.7°	41.8 yrs., + 0.43	1990 yrs.
Monozygotic twinning	0.023	1.3°	0.025	1.4°	18.1 yrs., − 0.21	3670 yrs.

* Only the first three digits of the entries in this column are significant figures.

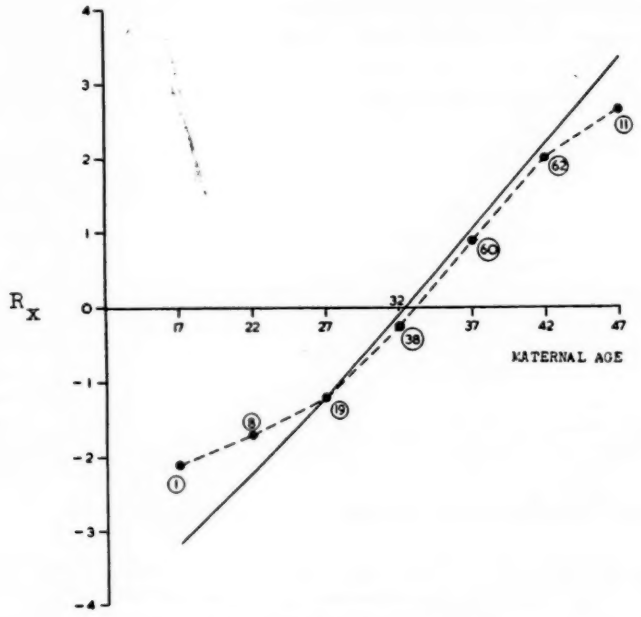
has been omitted from Table 3. Since there is no evidence of a significant difference in variances between the maternal age distribution of achondroplasics, on the one hand, and normal individuals, on the other, the conditional equation was taken to be linear for this character. The tangential equation for achondroplasia suggests an effect somewhere midway between that of mongolism and dizygotic twinning.

The steepness with which a parabola rises is indicated, to some extent, by the length of its latus rectum. On the whole, the longer the latus rectum the more gradually does the parabola increase its slope as it rises. The latus rectum, in itself however, is not characteristic since we need to know also the position of the vertex. Thus a condition whose parabola has a distant focus (a short latus rectum) will not really

be much influenced by maternal age if the vertex lies in the age interval when women are most fertile. On the other hand, a condition, such as mongolism, which does not appear to have a very steep parabola, still produces the most marked effect for, graphically, the vertex (with the exception of one series) is well before the age at which the human reproductive period begins.

The abscissa-coordinate of the vertex is particularly interesting, since it corresponds, for all the characters except monozygotic twinning, to the theoretical maternal age at which the probability of the character is lowest. It is, of course, meaningless to extrapolate a maternal age effect beyond or before the reproductive period. It is impossible to say what sort of infant an eight-year-old might produce, while the negative ages are obviously just analytic numbers. Still, the very fact that the theoretical point of minimum probability seems to fall outside the range is itself of interest.

We note that in only one case does the minimum lie close to the population mean.

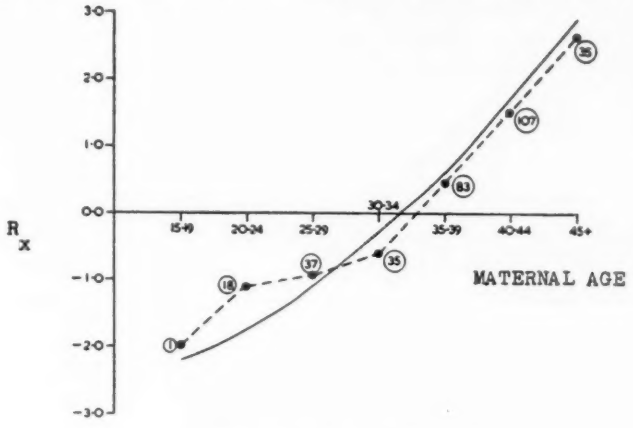


GALTON LABORATORY–MONGOLISM
199 CASES

LOCUS OF CONDITIONAL EQUATION
OBSERVED VALUE OF R_x
NUMBERS OBSERVED SHOWN IN CIRCLES

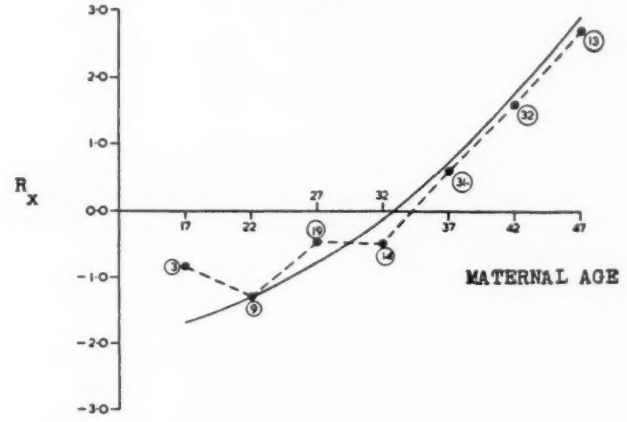
FIG. 1

In congenital heart disease—where the population mean is 27.33—the two maternal ages fall in the same interval. With the exception of this condition, the findings presented here are somewhat reminiscent of the work of Karn and Penrose (1951) on birth weight and gestation time, where mean and optimum—with respect to survival



VAN DER SCHEER – MONGOLISM
316 CASES

FIG. 2

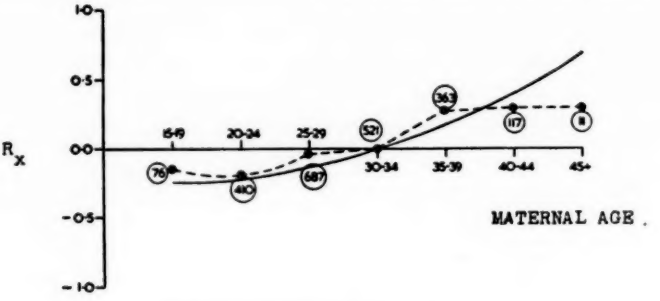


BEALL & STANTON MONGOLISM
121 CASES

LOCUS OF CONDITIONAL EQUATION.
OBSERVED VALUE OF R_x
NUMBERS OBSERVED SHOWN IN CIRCLES.

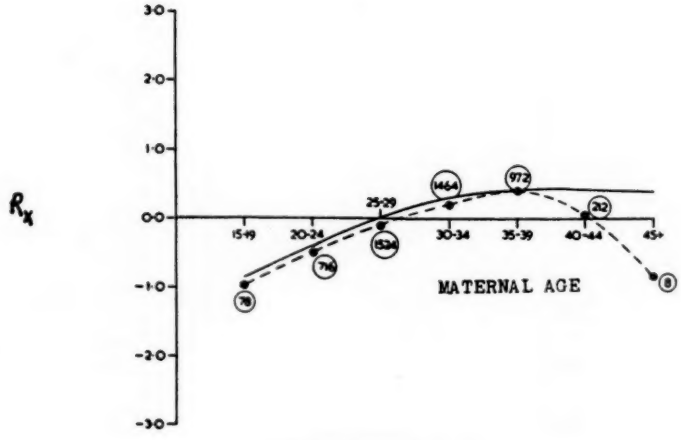
FIG. 3

—were markedly discrepant. It is not certain whether twinning can be considered a deleterious or an advantageous character, but the other conditions are clearly harmful to the persons who are affected with them, and almost certainly diminish fertility. Note that the monozygotic twin series is the only one which leads to a negative parabola (i.e. a parabola facing downwards). The "U-shaped" maternal age effect shown in the graph of the congenital heart data is probably not peculiar to congential heart disease. It is probable that had a study on stillbirths been undertaken by this



MONOZYGOTIC TWINS
2185 MATERNITIES

FIG. 4

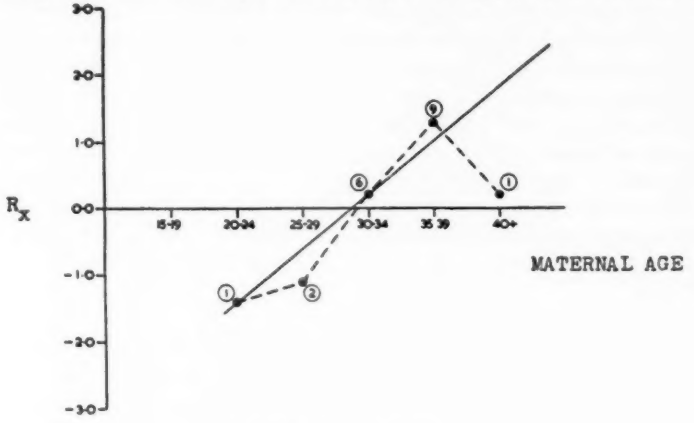


DIZYGOTIC TWINS
4974 MATERNITIES

——— LOCUS OF CONDITIONAL EQUATION
●- - -● OBSERVED VALUE OF R_x
NUMBERS OBSERVED SHOWN IN CIRCLES.

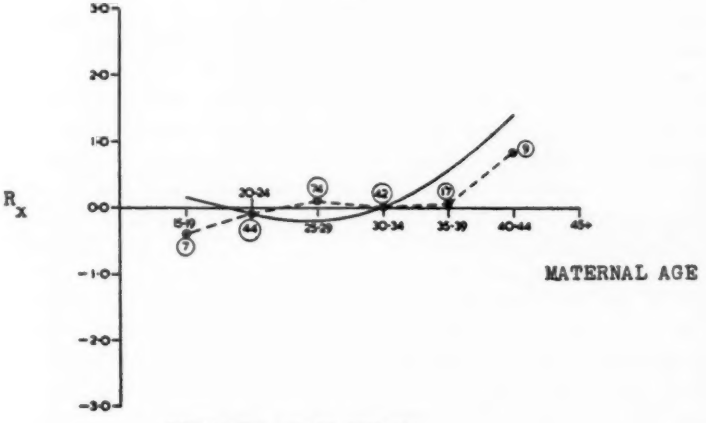
FIG. 5

method, a similar phenomenon would have been observed after eliminating the influence of order of birth. Yerushalmy has published a number of graphs—based on extensive U.S.B.R.A. statistics—which are highly suggestive. Unfortunately, Yerushalmy's work also indicates considerable heterogeneity in the influence of maternal age on stillbirths rates, and it may be, as he suggested, that here the action



ACHONDROPLASIA – 19 CASES

FIG. 6



CONGENITAL HEART DISEASE
193 CASES

LOCUS OF CONDITIONAL EQUATION.
OBSERVED VALUE OF R_x
NUMBERS OBSERVED SHOWN IN CIRCLES.

FIG. 7

of order of birth and maternal age is not independent and that interval between successive pregnancies is of considerable importance.

In Figures 1 through 7 several of the series have been graphed. The log ratio, R_x, is indicated along the ordinate in each case. The midpoints of the maternal age intervals (or the intervals themselves) are shown along the abscissa. Each particular value of R_x is indicated by a dot and the dots are connected with a broken line. The information factor—the number observed in the affected group—is enclosed in a circle above or below the appropriate vale of R_x. The conditional equation has been plotted to the same scale as R_x, and is indicated by a smooth black line. The reader can thus roughly appraise the goodness of fit in each case.

An exact comparison of the 'expected' value of R_x, obtained from the conditional equation, and the observed value, obtained from the raw data, is laborious and will not be undertaken here. The graphs suggest a reasonably good fit in the case of mongolism, congenital heart disease, and achondroplasia (although the series is very small), while the fit for the twinning data seems considerably rougher.

D. Criticisms

There are at least two criticisms which can be made of the above method of analysis. First, the assumption that the distributions are Gaussian is only very roughly true. The distribution of maternal ages in national birth populations has a positive skew, while in the case of mongolism, several series show a tendency towards bimodality. Still, as the reader can see from the series graphed, most of the data give a fairly good fit.

The second possible criticism is more subtle. It may be that the mothers of mongols are genetically different from women in the general population, and, as a result of their genotype, tend to reproduce abnormally late in life. This suggestion was put forward by Penrose (1951). If such a hypothesis were true, although the methods used above would still disclose the incidence (or a quantity proportional to it) at each maternal age, they would not convey any information about the frequency of mongol children at each maternal age among all children at that age who come from "contributive-type" mothers. This latter incidence, if Penrose's hypotheses were true, would clearly be of far greater interest. There is, however, evidence to suggest that any delayed or extended reproductivity that may exist among the mothers of mongols is slight. If the "contributive-type" women had their reproductive period delayed until late in life, an excess of early born mongols (i.e. mongols of low birth rank) at late maternal ages would be expected. If, on the other hand, the period of reproduction were merely extended, there would be too few early born mongols from old mothers (and to many late born ones). Thus far, neither of these effects has been observed. Nonetheless the suggestion can by no means be ruled out.

II. THE 'IMPORTANCE' OR 'STRENGTH' OF A MATERNAL AGE EFFECT

A. Methods

Maternal age is a continuous variable, and most of the characters with which maternal age is supposed to be associated are discontinuous. Therefore the measurement of the degree of association between maternal age, on the one hand, and the

presence or absence of some character, on the other, constitutes a rather special problem in descriptive statistics, as does the demonstration of the measurement of association between two discontinuous characters, such as order of birth and, say, mongolism—a problem to which the index about to be proposed is equally applicable. Not many investigators have concerned themselves with measuring the degree or extent to which maternal age influences the production of one or more abnormalities. The biserial form of the product moment correlation coefficient is the only index which has been used (Wright 1926, 1934), although the statistical significance of the maternal age effect has sometimes been employed as a crude measure of the strength of maternal age in the aetiology of a condition. The biserial is an elegant index, but it has two disadvantages. First, its real meaning is a little difficult to grasp, its derivation being rather oblique. Secondly, it is dependent upon the total incidence of the character being studied, and for the very rare characters with which most maternal age studies are concerned, the biserial tends to be small and intractable.

Another, less elegant, index will now be proposed.

Consider a table where we have a discontinuous character, such as mongolism or normality and a continuous one such as maternal age. Let us represent the various maternal ages (or maternal age intervals) as $x_1, x_2 \cdots x_i \cdots x_k$.

Let P_{M_i} = the proportion of abnormals occurring at maternal age i.

Let P_{N_i} = the proportion of normals occurring at maternal age i.

Let $d_i = P_{M_i} - P_{N_i}$.

Our table looks like this:

				Totals
Maternal age	x_1	x_2	$\cdots x_k$	All ages
Abnormals	P_{M_1}	P_{M_2}	$\cdots P_{Mk}$	1
Normals	P_{N_1}	P_{N_2}	$\cdots P_{Nk}$	1
Difference	d_1	d_2	$\cdots d_k$	0

The index proposed is defined simply as $I_{AD} = \frac{1}{2} \Sigma \mid d_i \mid$, where for I_{AD} one reads "the index of absolute differences".

The interpretation of this index is fairly simple. We may regard the sample of abnormals as a group comprised of two different kinds of children: those who seem to owe their condition, at least partially, to the increased (or decreased) age of their mothers, and those who do not. It is easy to show that I_{AD} is an estimate of the proportion of children falling into the former category. Since $\Sigma\, d_i = 0$, $\frac{1}{2} \Sigma \mid d_i \mid$ must always equal the sum of the positive values of d_i. Therefore I_{AD} must equal the proportion of abnormal children who are born at each maternal age over and above the expectation for normal children. Thus, we may say that I_{AD} estimates the proportion of affected children who seem to owe their affection, at least partially, to the increased (or decreased) age of their mothers. Notice that I_{AD} will equal one only if all the affected children occur at a unique maternal age.

Geometrically, I_{AD} corresponds to the total area which lies above the ordinates of the distribution of normals and below the ordinates of the distribution of abnormals.

It is interesting finally to note that I_{AD} will measure the strength of association even when the maternal age effect is not perfectly consistent. For example, if birth

at a very young or a very old maternal age increases the probability of affection with some abnormality, the difference between the proportions P_M and P_N will be correspondingly increased at the ends, and decreased in the middle, of the range. But since I_{AD} depends upon the absolute differences, it will still 'catch' the effect. The biserial, however, is proportional to the difference between the means divided by the square root of the total variance of maternal age. A near equal effect at both ends of the range would tend, by cancelling itself out, to render the difference between the means very small, while increasing the total variance. Therefore as such an effect becomes *more* pronounced; the biserial would tend to zero, while I_{AD} would tend to one. I_{AD}, as can be seen, makes no *a priori* assumptions about the distribution of maternal age in each group.

The chief disadvantage of the index proposed here is that its sampling variance seems extremely difficult to derive, and the index is therefore primarily of descriptive value. In the computations which follow, the exact variance of I_{AD} will not be given.

However, Dr. C. A. B. Smith of University College, London has pointed out to me that an approximate variance can be obtained by the following argument: We may classify values of d_i into three groups:

1. $d_i > 0$
2. $d_i < 0$
3. $d_i = 0$

Values of d_i close to zero contribute very little to I_{AD}, and for purposes of finding an approximate variance, we may restrict our attention to those values of d_i which are sufficiently large that they fall into group 1 or into group 2 with probability nearly one.

In the case of group 1, $| d_i | = (P_{Mi} - P_{Ni})$ and has variance

$$\frac{P_{Mi}(1 - P_{Mi})}{M} + \frac{P_{Ni}(1 - P_{Ni})}{N} - 2 \operatorname{cov} (P_{Ni} P_{Mi})$$

where M and N are the respective total number of normals and abnormals. Since in all cases to be considered in this paper N is extremely large compared with M, we may approximate the variance of $| d_i |$, $d_i > 0$, by $\frac{P_{Mi}(1 - P_{Mi})}{M}$. The covariance term and the variance of P_{Ni} are of the order N^{-1} or less, and are therefore dropped.

Turning to group 2, we find $d_i = -(P_{Mi} - P_{Ni})$, which, by the same reasoning, has approximate variance.

$$\frac{P_{Mi}(1 - P_{Mi})}{M}$$

Hence the variance of $\Sigma | d_i |$ is approximately

$$\Sigma \frac{P_{Mi}(1 - P_{Mi})}{M} + 2 \sum_{i \neq j} \operatorname{cov} | d_i | | d_j | .$$

For simplicity we take the summation over all values of i, rather than merely over those which have d_i values in groups 1 and 2. The error so introduced is on the safe side, that is, it tends to result in an *overestimate* of the variance. The covariance of $|d_i| \, | \, d_j \, |$ is

$$\pm \frac{P_{Mi} P_{Mj}}{M} + 0(N^{-1}),$$

The $\pm$ sign being taken in the negative sense when d_i and d_j are of the same sign and in the positive sense when they are of different sign.

Although some of the covariance terms are going to be positive and others negative, we shall continue to err on the safe side, if we consider all covariances to be positive, and write

$$\text{var } \Sigma \, | \, d_i \, | < \frac{\sum P_{Mi}(1 - P_{Mi}) + 2 \sum_{i \neq j} P_{Mi} P_{Mj}}{M} + 0(N^{-1})$$

from which it follows that var $\Sigma \, | \, d_i \, | < \frac{2}{M} + 0(N^{-1})$. Since $I_{AD} = \frac{1}{2}\Sigma \, | \, d_i \, |$, var $I_{AD} < \frac{1}{2M} + 0(N^{-1})$, and we shall consider $\frac{1}{2M}$ to be an approximate variance of I_{AD}. This variance may in general be used to measure the precision of I_{AD} as an estimate, provided there is *a priori* knowledge, based on an ordinary significance test, that I_{AD} differs significantly from zero, and provided $\frac{1}{N}$ is small compared with $\frac{1}{M}$.

Whenever I_{AD} values (in per cent) are given in the tables followed by a plus or minus sign, the approximate standard error (times 100) will be placed after them in brackets.

B. Analysis of Data

In Table 4 the indices of absolute differences are set out for all the series analyzed earlier. (The control series used in each case is the same as the one specified in Table 1.) The indices are also given for two other series: the data of Mørch (1941) on 99 sporadic cases of achondroplasia and Buchi's (1950) series of 172 cases of congenital heart disease. Mørch compared his dwarfs with a large multiennial Danish birth population, which was chosen to cover, as nearly as possible, the years of birth of the affected. Buchi studied a Copenhagen birth population comprised of 167,940 children among whom were 172 cases of congenital heart disease. Buchi did not tabulate his data very fully, and the I_{AD} had to be computed from the ratio of observed to expected at each maternal age. The chi-squares which Buchi and Mørch give for the comparison of their respective data with the corresponding control are highly significant.

An inspection of Table 4 discloses that the association between maternal age and mongolism is stronger than the association between maternal age and any of the other conditions. Dizygotic twinning and achondroplasia rank next. Monozygotic twinning and congenital heart disease would seem to show the weakest effects.

TABLE 4

Character	Series	Code Number†	No. of Cases	I_{AD}(%)	$100I_{AD/55.1}$
Mongolism	Penrose	1	260	55.1 ± (4.4)	100.0
	Van der Scheer	2	316	47.6 ± (4.0)	86.4
	Orel	3	104	40.6 ± (6.9)	73.7
	Beall and Stanton	4	121	43.3 ± (6.4)	78.6
	Hanhart	5	52	46.1 ± (9.8)	83.7
	Galton Laboratory	6	199	49.7 ± (5.0)	90.2
Achondroplasia	Mørch (1941)	7	99	17.7 ± (7.1)	32.1
	Galton Laboratory	8	19	43.3 ± (16.2)	78.6
Congenital heart disease	Buchi (1950)	9	172	2.8 ± (5.4)	5.1
	Galton Laboratory	10	193	6.0 ± (5.1)*	10.9
Dizygotic twinning	McArthur (1949)	11	4974	12.6 ± (1.0)	22.9
Monozygotic twinning	McArthur (1949)	12	2185	5.4 ± (1.5)	9.8

* After correction for an independent parity effect.
† For use in interpreting figure 2.

Fairly marked discrepancies between two series are noticeable in the case of achondroplasia and congenital heart disease. Among the achondroplasics, this may be in part due to the fact that the Galton Laboratory series is probably more heterogeneous than Mørch's series. The Galton data include one partial achondroplasic and one case where the dwarfism seems to be a symptom of the Ellis and van Creveld (1940) syndrome. Among the congenital heart children, it should be noted that the index for the Galton Laboratory data was computed *after* the distribution of maternal ages was standardized for an observed primogeniture effect. Buchi did not standardize his data for order of birth. Since congenital heart disease shows a consistent late maternal age effect, one might reasonably expect the I_{AD} computed from Buchi's series to be smaller.

In the last column of Table 4 the indices have been divided by the largest index (from Penrose's mongol series) and multiplied by one hundred. The figures in this column are intended to give the reader some idea of the strength of the various maternal age effects relative to the effect for mongolism (and like the indices themselves they also reveal the heterogeneity between the different mongol series). Since the influence of maternal age in the causation of mongolism seems more marked than the influence of maternal age in the causation of any other condition thus far reported in the literature, standardizing the indices for the maternal age effect in mongolism

RELATIVE STRENGTHS OF SEVERAL MATERNAL AGE EFFECTS

FIG. 8

seems a meaningful procedure, enabling one to calibrate maternal age effects on a scale in which the maternal age effect of mongolism ranks as 100 or close to it. The standardizing index was taken from Penrose's data, simply because this index is the largest. Perhaps the correct index for mongolism is slightly less.

In Figure 8 the indices are shown graphically, as segments along a line. The position of each series on the line is shown by a number given to the series in the column headed 'Code Number' in Table 4. Penrose's mongol data are represented as a segment 100 units long.

III. SUMMARY

Some problems arising in the representation of maternal age effects are discussed. Methods for describing the way in which the incidence of an abnormality changes with maternal age are given, and a new index for measuring the strength of association between probability of affection and maternal age is derived. Several series of cases are analyzed.

ACKNOWLEDGMENT

I originally derived the conditional equation by assuming equal variances. I am grateful to Professor L. S. Penrose, F.R.S., for showing me that a better fit could be obtained by taking differences in variance into account.

Appendix

Condition	m_2	m_1	V_2	V_1
Mongolism (Penrose)	37.06	28.63	48.02	33.92
Mongolism (Van der Scheer)	37.16	29.94	46.14	36.02
Mongolism (Orel)	34.93	29.07	53.07	32.34
Mongolism (Beall & Stanton)	35.64	28.39	59.17	41.35
Mongolism (Hanhart)	36.23	29.52	32.62	31.57
Mongolism (Galton Lab.)	36.50	28.63	36.35	33.92
Achondroplasia	33.84	28.63	20.73	33.92
Congenital Heart Disease	28.16*	27.33*	30.80*	23.09*
Dizygotic Twinning	30.22	28.63	29.85	33.92
Monozygotic Twinning	29.47	28.63	36.63	33.92

* Corrected for independent parity effect.

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Estimation of the Frequency of Partially Sex-linked Genes in Man*

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INTRODUCTION

In 1936 HALDANE published the results of his researches on a new type of human inheritance, which was designated as incomplete sex-linkage or partial sex-linkage. Based on these results, he constructed provisional maps of sex-chromosomes on which the six genes responsible for the abnormalities apparently showing this type of inheritance, namely, total color-blindness, Oguchi's disease, xeroderma pigmentosum, epidermolysis bullosa dystrophica and dominant and recessive types of retinitis pigmentosa, were located. Subsequently, several other genes were added to the list.

The problem was attacked from the theoretical standpoint also. Haldane and Moshinsky (1939) pointed out that in the diseases caused by partially sex-linked genes there should be a moderate excess of affected daughters among the children from consanguineous marriages. Macklin (1952) carried the matter further with special reference to: (1) the proportions of the four types of first cousin matings; (2) the site of the mutant gene on the sex-chromosomes of a common ancestor of the cousins; and (3) the crossover value of the gene in question.

It is known that the frequency of a rare recessive gene cannot be estimated from the trait frequency alone, owing to the interference of the factor of inbreeding. The estimation may be more effectively carried out on the basis of the incidence of first cousin marriages in the general population and among the parents of affected individuals. In partial sex-linkage the trait frequency depends not only on the gene frequency, but also on the factors pointed out by Macklin.

These relations seem to have been overlooked by many authors. The frequency of partially sex-linked recessive genes has been calculated in the same way as that of the autosomal recessive genes, for instance, for total color-blindness and xeroderma pigmentosum by Neel et al. (1949) and Oguchi's disease by Kawakami (1933).

This paper presents a detailed discussion of the effect of first cousin marriages on the frequency of homozygosis for a partially sex-linked recessive gene. It presents, also, equations which permit a more accurate estimation of the frequency of a partially sex-linked recessive gene from data concerning first cousin marriages.

GENE FREQUENCY AND FOUR TYPES OF COUSIN MARRIAGES

According to Weinberg (1920) and Dahlberg (1948) the frequency of an autosomal recessive gene may be given by the following formula:

$$k = \frac{c\{(r/16) + (15r^2/16)\}}{c\{(r/16) + (15r^2/16)\} + (1 - c)r^2} \qquad (1)$$

Received December 13, 1954.

* Contributions from the National Institute of Genetics, Japan, No. 86.

which becomes:

$$k = (c + 15rc)/(c - rc + 16r),$$

or, restated in the terms of r,

$$r = \frac{c(1 - k)}{16k - 15c - ck},$$

where r = the frequency of the recessive gene responsible for the trait in question,
k = the proportion of first cousin marriages among the parents of affected individuals, and
c = the proportion of first cousin marriages among the population as a whole.

In formula (1) $r/16$ denotes the probability of meeting of the mutant genes from a common ancestor and $15r^2/16$ the probability of the same gene derived from different ancestors meeting each other, and the sum $r/16 + 15r^2/16$ corresponds with the probability of obtaining a homozygous child from a first cousin mating.

In partial sex-linkage these values are variable depending upon the crossover value and the type of cousin marriages. Macklin has given the formulas denoting the probability of both members of a cousin couple possessing the gene derived from their common ancestor. According to her, the probability in the mating in which the couple are the children of brothers (type 1) is $pq(1 + 2p^2 + 2q^2)/2$; that in the mating of the children of sisters (type 2), $(1 + 2p^2 + 2q^2)/8$; that in the mating of a son of a brother and a daughter of a sister (type 3), $p(1 + 4q^2)/4$; and that in the mating of a son of a sister and a daughter of a brother (type 4), $q(1 + 4p^2)/4$; where p corresponds to the crossover value of the gene in question and $q = 1 - p$.

If these probabilities are divided by 4, we shall obtain the probabilities of getting an affected child from each type of cousin mating, when one of the common grandparents of the cousins had the gene in question. These values will be designated as d_1, d_2, d_3 and d_4 respectively. Then the probability of getting an affected child from a cousin marriage of type 1 is: $d_1r + r^2(1 - d_1)$ or $d_1r(1 - r) + r^2$. Thus, we find:

$$k = \frac{\sum_{i=1}^{4} [d_i c_i r(1 - r) + c_i r^2]}{\sum_{i=1}^{4} [d_i c_i r(1 - r) + c_i r^2] + (1 - c)r^2},$$

where c_1, c_2, c_3 and c_4 are the incidences of the types 1–4 of cousin marriages respectively in the population in question.

Since $c = c_1 + c_2 + c_3 + c_4$, the formula becomes:

$$k = \frac{(1 - r)\sum_{i=1}^{4} d_i c_i + cr}{(1 - r)\sum_{i=1}^{4} d_i c_i + r},$$

or, restated in the terms of r,

$$r = \frac{(1 - k)\sum_{i=1}^{4} d_i c_i}{(1 - k)\sum_{i=1}^{4} d_i c_i + k - c} \tag{2}$$

Thus, the estimation of the gene frequency in partial sex-linkage should be carried out by taking the crossover value and the frequency of the four types of cousin marriages into consideration.

The values of d_1, d_2, d_3 and d_4 for various crossover frequencies are presented in Table 1. As shown in the table, the d_2 value is always larger than $\frac{1}{16}$ or 0.0625, while the d_1 and d_3 values are always smaller than $\frac{1}{16}$, as long as the crossover value is smaller than 0.5. The values for d_4 are relatively constant, having a minimum value of approximately 0.0579 at a crossover frequency of 0.167 and a maximum value of 0.0625 at crossover frequencies of 0.0 and 0.5.

Macklin assumed in her calculation that the four types of cousin matings occur in equal frequency. If this is the case,

$$(1 - k)\sum_{i=1}^{4} d_i c_i = (1 - k)\tfrac{1}{4}c(d_1 + d_2 + d_3 + d_4)$$

$$= (1 - k)cd$$

TABLE 1.—PROBABILITIES OF AFFECTED CHILDREN FROM EACH TYPE OF COUSIN MATINGS*

Cross-Over Value	Type 1 $d_1 = \frac{pq(1 + 2p^3 + 2q^3)}{8}$	Type 2 $d_2 = \frac{(1 + 2p^3 + 2q^3)}{32}$	Type 3 $d_3 = \frac{p(1 + 4q^3)}{16}$	Type 4 $d_4 = \frac{q(1 + 4p^3)}{16}$	Mean $d = \frac{(d_1 + d_2 + d_3 + d_4)}{4}$
0	0	0.0938	0	0.0625	0.0391
0.05	0.0167	0.0878	0.0144	0.0600	0.0447
0.10	0.0297	0.0825	0.0265	0.0585	0.0493
0.15	0.0397	0.0778	0.0365	0.0579	0.0530
0.20	0.0472	0.0738	0.0445	0.0580	0.0559
0.25	0.0527	0.0703	0.0508	0.0586	0.0581
0.30	0.0567	0.0675	0.0555	0.0595	0.0598
0.35	0.0594	0.0653	0.0588	0.0605	0.0610
0.40	0.0612	0.0638	0.0610	0.0615	0.0618
0.45	0.0622	0.0628	0.0622	0.0622	0.0623
0.50	0.0625	0.0625	0.0625	0.0625	0.0625

* The relationships of the wife to her husband are as follows:
Type 1. She is his father's brother's daughter.
Type 2. She is his mother's sister's daughter.
Type 3. She is his father's sister's daughter.
Type 4. She is his mother's brother's daughter.

where $\frac{1}{4}(d_1 + d_2 + d_3 + d_4) = d$, and formula (2) reduces to:

$$r = \frac{dc(1 - k)}{dc(1 - k) + k - c} \tag{3}$$

The values of d are also presented in Table 1.

In the case of autosomal recessive genes, all the values of d_1, d_2, d_3 and d_4 are equal to $\frac{1}{16}$, therefore the term $\Sigma d_i c_i$ in formula (2) becomes $\Sigma c_i/16$ or $c/16$, and formula (2) becomes:

$$r = c(1 - k)/(16k - 15c - ck),$$

which is identical with formula (1).

NUMERICAL EXAMPLE

The formulas mentioned above will be applied to the data of total color-blindness.

Macklin (1952) tabulates the literature where the types of cousin matings among Caucasian are reported. She has found that, with the exception of Orel's data, there is no significant deviation from the equality of the four types. Of the Japanese population, however, this does not seem to be true. According to Morton and Schull (unpublished), the four types of cousin marriages occurred in the proportions of 21.6 per cent (type 1), 33.2 per cent (type 2), 18.4 per cent (type 3) and 26.7 per cent (type 4) respectively among the 689 cases collected from the material of the Atomic Bomb Casualty Commission. This material is derived from three cities in the western part of Japan and may not adequately represent the general Japanese population. The present author has made special enquiry through his friends residing in various parts of Japan for the actual instances of cousin matings. The data are presented in Table 2. The sum totals at the bottom of the table indicate that the distribution of the different types of cousin matings are nearly in accord with the proportions given by Morton and Schull and does not conform to the expectation of equal incidence, the proportion of type 2 being significantly higher and that of type 1 being lower. The following calculation will be carried out under the assumption that the four mating types occur with equal frequency among Caucasian, whereas they occur in the proportions shown in Table 2 among Japanese.

Following Haldane (1936), the crossover value of the gene responsible for the total

TABLE 2.—THE PROPORTIONS OF THE FOUR TYPES OF COUSIN MARRIAGES AMONG JAPANESE

Region	Type 1	Type 2	Type 3	Type 4	Total
Kyusyu	24	36	22	33	115
Sizuoka & Yamanasi	5	10	5	14	34
Okayama	21	43	31	34	129
Ina	11	17	15	20	63
Others	7	10	9	7	33
Total	68	116	82	108	374
Per cent	18.2	31.0	21.9	28.9	100.0

color-blindness is considered to be 8–10 per cent. Fisher (1936) applied tests of significance to Haldane's data and obtained the recombination values 8.12 assuming complete ascertainment, 14.40 assuming single ascertainment and 20.57 by using the ancillary information. The present author analyzed the data on this abnormality collected from the Japanese literature and obtained crossover values by the indirect method similar to Haldane's and Fisher's estimates. The detailed statement will be deferred to a future publication. In the following calculation the figure of 15 per cent will be used as the crossover value of the gene for total color-blindness.

Neel et al. give estimations of the frequencies in European and Japanese populations of the recessive genes responsible for five diseases including total color-blindness by using formula (1). They have assumed c, the incidence of cousin marriages in the general population, as 0.01 for European and 0.06 for Japanese. These values will be used in this paper, since they seem to be rather reasonable estimates.

For the value k, the incidence of cousin matings among parents of the affected, Neel et al. have used 0.11–0.21 for Caucasian and 0.39–0.51 for Japanese. These figures will be used here for the sake of convenience of showing how their estimates will be affected by the consideration of partial sex-linkage.

When these values for Japanese population are put into formula (2), we obtain 0.0036–0.0062 for the frequency of the gene for total color-blindness. This value is about 12 per cent lower than the value given by Neel et al.

For Caucasians, since the four mating types are assumed to occur in the same frequency, we can use formula (3) and obtain the value 0.0021–0.0047, which is 85 per cent of Neel et al.'s estimates.

EFFECTS OF PROPORTIONS OF THE FOUR MATING TYPES

As stated above, when the four types of cousin marriages occur in the same frequency, the gene frequency may be given by formula (3). Let r and r_0 designate the two estimates of the frequency of a given gene under the assumptions of partial sex-linkage and autosomal recessive inheritance, respectively. Let d and d_0 designate the mean probabilities of getting affected children from cousin matings under these same assumptions. Then the ratio r to r_0 is:

$$\frac{dc(1-k)}{dc(1-k)+(k-c)} \cdot \frac{d_0c(1-k)+(k-c)}{d_0c(1-k)}.$$

From (3) it can be shown that $(k-c)/dc(1-k) = (1-r)/r$. Hence, if r is small (say less than 0.01) $k-c$ is 100 or more times as large as $dc(1-k)$ or $d_0c\,(1-k)$. Therefore, the ratio of r to r_0 for rare genes may be regarded as equal to d/d_0. If $d_0 = \frac{1}{16}$ as in the case of an autosomal recessive gene, r/r_0 will be approximately equal to $16d$. This value will correspond to the multiplier for correction. If the estimate calculated under the assumption of autosomal recessiveness is multiplied by this value, we shall obtain the frequency of the partially sex-linked gene.

The actual figures of $d \times 16$ are presented in Table 3. For example, when Neel's estimate for Caucasian, 0.0025–0.0055, is multiplied by 0.847, which is the multiplier for correction at a crossover value of 15 per cent, the figure 0.0021–0.0047 is obtained: the same figure as that obtained by using formula (3).

TABLE 3.—RATIOS OF PROBABILITIES OF GETTING AFFECTED CHILDREN FROM COUSIN MATINGS IN PARTIAL SEX-LINKAGE TO THOSE IN AUTOSOMAL RECESSIVENESS

Crossover Value	$d \times 16^*$
0	0.625
0.05	0.715
0.10	0.789
0.15	0.847
0.20	0.894
0.25	0.930
0.30	0.957
0.35	0.976
0.40	0.989
0.45	0.997
0.50	1.000

* The values are calculated assuming equal frequency of the four types of cousin matings.

Table 3 indicates that the stronger the linkage, the greater is the difference between the frequencies of autosomal and partially sex-linked genes, under the same k and c values. If the crossover value is higher than 30 per cent, the difference may be so small as to make the correction almost unnecessary. It will be noticed in the table that, when the four mating types occur with equal frequency, the estimated gene frequency is always lower in the case of partial sex-linkage than in the case of autosomal recessiveness, if k and c values are the same.

However, if the proportions of the different types of cousin matings deviate considerably from equality, the situation may be changed. If the proportion of matings in which the husband and wife are related through their mothers (type 2, Table 1) exceeds 25 per cent, the gene frequency will approach the value found in the case of autosomal recessives. If the crossover frequency is low and the proportion of type 2 cousin marriages is much higher than 25 per cent, the computed gene frequency may exceed the value found in the case of autosomal recessives with the same values of k and c. Contrariwise, if the proportion of matings in which the husband is related to his wife through his father (types 1 and 3, Table 1) is high, the gene frequency will be still lower than in the case of equal incidence of the four types.

Kawakami (1933) has pointed out for Oguchi's disease the tendency that male patients are more apt to consult doctors than female patients. If this is true, the cousins among the parents of the affected persons in the literature could not be regarded as a random sample taken from all the cousins among the parents of the affected. The detailed discussion on this point will be deferred to a subsequent paper.

SUMMARY

When a gene is recessive and partially sex-linked, the estimation of its frequency should be carried out under consideration of several factors, which are specific to partial sex-linkage: (1) the crossover value of the gene; (2) the incidence of the four types of cousin marriages; as well as some factors which are common to all rare recessive genes, either autosomal or partially sex-linked: (3) proportion of cousin marriages in the population as a whole and (4) that among parents of the affected.

Formulas for the estimation of the frequency of partially sex-linked genes and the figures for the calculation are given. It has been shown that the stronger the linkage, the greater is the difference between the frequencies of an autosomal recessive gene and of a partially sex-linked gene, even if the proportions of the cousin marriages among parents of the affected are the same. For instance, the frequency of the gene responsible for total color-blindness under the assumption of partial sex-linkage is about 15 per cent lower than the frequency calculated under the assumption of autosomal recessiveness.

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Hand Prints and Handedness

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ASCERTAINMENT OF the roles of heredity in bringing about common nonpathological variations in human behavior entails difficulties not so likely to be encountered elsewhere. Concentrations of the occurrence of an uncommon variation within certain families is usually accepted as an indication that it may be inherited. But correlations between relatives with respect to a behavioral variation may also be used as evidence for the role of environment, for the child may have learned the behavior by association with and imitation of other members of the family. Gene frequency analysis may be employed to test simple modes of inheritance, but agreement between observed and expected frequencies of a trait does not necessarily prove its inheritance. It may be happenstance.

Perhaps the most convincing evidence of a genetic basis for a behavioral trait is to establish an association between it and a highly heritable physical variation which develops during fetal life, and which is not altered by postnatal circumstances. Such an association would indicate beyond reasonable doubt that the behavioral trait was influenced by heredity. There are at least two ways in which two hereditary traits may be associated. If they are due to two linked gene loci, one variant of trait A will go together with a certain variant of trait B in some families; but in other families the association will be in the opposite sense, and the population as a whole will show no association.

The second type of association results from the affects of one or more genes on both traits, or pleiotropy. Under these circumstances the correlation is always of the same type, and is evident in whole populations, as well as within families.

Handedness is a common behavioral variation, concerning which the importance of heredity is a topic of considerable controversy. There is no doubt that in many instances training can bring about a change. Europeans eat left-handed, whereas most Americans eat right-handed, yet they are of somewhat the same ethnic stock. Many left-handed children have been trained to write with their right hands. But the observation that handedness may sometimes be altered by no means rules out the possibility that heredity is also important.

There is a highly significant correlation between family relationship and similarities in handedness. In families where both parents are left-handed, about 50% of the children are left-handed; in families where only 1 parent is left-handed, about 17% of the children are left-handed; and in families where both parents are right-handed, only 6% of the children are left-handed (Rife, 1940). But as I have already pointed out, these correlations may be presented as evidence for either heredity or

Received January 24, 1955.

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environment. They do indicate, however, that left-handedness is not due to a simple pair of recessive genes.

Intrapair differences, or "mirror-imaging," in handedness occur in approximately 20% of monozygotic twins. Newman, Freeman, and Holzinger, (1937) believed this phenomenon to be the result of relatively late separation of the embryos. This has not been substantiated by later findings (Rife, 1950), as there is little if any correlation between "mirror-imaging" in various traits in monozygotic twins, such as direction of hair whorls, dermatoglyphics, dentition, and handedness. Why, then, do some pairs show these differences, while others do not?

Investigations of the families of monozygotic twins, as well as those of dizygotic twins, have shown significantly higher frequencies of left-handedness among the immediate relatives of twins manifesting intrapair differences in handedness and pairs in which both members are left-handed, than among those of twins both of whose members are right-handed. It should be noted here that dizygotic twins show slightly more "mirror-imaging" in handedness than do monozygotic twins. A gene frequency analysis of over 500 pairs of twins revealed that the incidences of mirror-imaging in both types of twins agrees very closely to what would be expected if handedness is conditioned by a single pair of alleles lacking dominance, one homozygous condition resulting in right-handedness, the other in left-handedness, while the heterozygotes are ambidextrous. Those in the latter category are not predisposed either way, their handedness depending upon environmental factors. Mirror-imaging in monozygotic twins occurs in heterozygous pairs, possibly due to the operation of the asymmetry mechanism proposed by Newman. But whether monozygotic twins show the same or different types of handedness appears to depend upon the genotype of the twins rather than upon the period at which separation of the embryos occurred, (Rife, 1950).

Agreement with the gene frequency analysis does not necessarily prove that handedness is inherited in the manner proposed. It has also been noted (Verschuer, 1932) that observed frequencies in both types of twins agree closely with what one might expect on the basis of chance alone. If there were no data suggestive of heredity aside from family pedigrees and gene frequency analyses, one might be inclined to discount the importance of heredity. But there are associations between handedness and dermatoglyphics which present convincing evidence for a genetic basis for functional handedness.

The dermatoglyphic configurations on palms and fingertips are fully established after 18 weeks of fetal development, and are not altered thereafter. Moreover, they are highly heritable, and manifest tremendous variations from one individual to another. Any significant associations between dermatoglyphics and handedness thus appear to rule out any possibility that handedness is determined entirely by post-natal environment and training. Rife (1943) obtained evidence from family data that genes affecting handedness are linked with at least 1 pair which determine whorls on finger-tips and patterns in the fourth interdigital area of the palm.

Various investigators (Newman, 1934; Bettman, 1932; Cummins, Leche, and McClure, 1931; Cummins, 1940; Cromwell and Rife, 1942; Rife, 1943) have noted that

associations characteristic of pleiotropy appear to exist between dermatoglyphics and handedness. In general, the hand prints of left-handers appear to possess more bilateral symmetry than do those of right-handers. That is to say, series of prints obtained from right-handers show greater total differences between right and left sides in the occurrence of patterns in the various areas than do those taken from left-handers. Although these observations are in general agreement, the differences are relatively small, of the order of from one to three percent. Obviously, very large numbers are needed to determine whether or not they are really significant. Unfortunately, most investigators have compared prints from only a few hundred individuals, whereas thousands are needed.

This report is concerned with the findings obtained from the prints of 3088 students at Ohio State University, who were students in an elementary genetics course. The investigation covered a period of approximately 10 years, and all students taking the course were required to provide data on handedness and dermatoglyphics. Patterns on the thenar/first interdigital area of the palm, and arches on the middle fingers were found to provide the best criteria of differences associated with handedness.

Several criteria were employed in the classification of handedness. These included preferred hand for throwing, writing, bowling, shooting marbles, tennis racket, driving a nail, sawing, whittling, and striking a match. Those definitely preferring the left hand for one or more of these operations were classed as left-handed. Undoubtedly many using the left-hand for only a few of these operations may be ambidextrous, but as we live in a right-handed world it is doubtful if any genotypic right-handers were included.

PATTERNS IN THE THENAR/FIRST INTERDIGITAL AREA

There are five areas on palms, on each of which patterns may or may not be present. Patterns consist of loops and whorls, and combinations of them. There are consistent and significant bimanual variations in the occurrence of patterns in each area. Patterns occur more frequently on right hands in the hypothenar, second, and third interdigital areas, whereas they occur more frequently on left hands in the thenar/first interdigital and fourth interdigital areas. These bimanual differences are greater in males than in females. Cromwell and Rife (1942) compared the prints of 600 right-handers with those of 753 left-handers, and found a trend towards reduction of bimanual differences, due chiefly to increases in patterns on the side having the lesser frequencies. This was found to hold true for all areas except the hypothenar, where the bimanual differences were the least of any of the areas. Although the trends were similar in each of the other four areas, they were only of the magnitude of approximately 1 to 2%. The same trend was noted for the occurrence of whorls on ring fingers, the finger characterized by highest frequencies of whorls. When the different areas were treated individually the differences were not statistically significant, but when pooled together the differences between right-handers and left-handers were significant.

The statistical significance of differences depends to some extent upon the ratios resulting from them. For example, an increase of from 1% to 3% is much more

likely to be statistically significant than of from 49% to 51%. Equal increases in percentages are more likely to indicate significant differences at low rather than at intermediate frequencies, providing equal numbers of individuals are used in both comparisons. Patterns occur with intermediate frequencies on the third and fourth interdigital areas, whereas they occur with comparatively low frequencies in the thenar/first and second interdigital areas. Of the latter areas the thenar/first is more satisfactory for purposes of comparison, partly because of the rarity of second interdigital patterns among Caucasians. A review of the findings of various investigators shows that the most consistent differences in total pattern frequencies occur in the thenar/first interdigital area. Inspection of Table 1 shows increases in the frequencies of patterns in the thenar/first interdigital area among left-handers, the only exception being in the relatively small sample of Keith, where a small insignificant decrease was noted. Totals of all six investigations show an increase of approximately 3% among patterns in left-handers, the difference between right- and left-handers being highly significant.

The figures in Table 1 do not tell whether the increases among left-handers are due to proportional increases on both sides or to increases on one side only. Table 2

TABLE 1.—DATA CONCERNING THE TOTAL FREQUENCIES OF PATTERNS IN THE THENAR/FIRST INTERDIGITAL AREAS OF RIGHT-HANDERS AND LEFT-HANDERS

Investigator	Numbers of Individuals		Patterns			
	Right-handers	Left-handers	Right-handers		Left-handers	
			No.	Percent	No.	Percent
Cromwell	600	740	122	10.1	180	12.1
Keith	79	86	24	15.2	23	13.4
Newman	100	100	14	7.0	25	12.5
Bettman	200	100	66	16.5	58	29.0
Cummins and Leche	300	244	33	5.5	49	10.1
Rife (this investigation)	2716	372	556	10.4	98	13.1
Total	3995	1642	815	10.20	433	13.17

$\chi^2 = 21.95$
$df = 1$
$p = < .000005$

TABLE 2.—THE DISTRIBUTION OF THENAR/FIRST INTERDIGITAL PATTERNS AMONG RIGHT-HANDERS AND LEFT-HANDERS

Handedness	Sex	Right Hand Only	Left Hand Only	Ratio Left to Right	Both	Total Number of Persons
Right	♂	13–1.08%	128–10.68%	9.8:1	68–5.64%	1198
Right	♀	19–1.25	106–6.98	5.5:1	77–5.07	1518
Right	♂ + ♀	32–1.17	234–8.61	7.3:1	145–5.30	2716
Left	♂	7–3.57	29–14.79	4.1:1	13–6.63	196
Left	♀	6–3.40	8–4.54	1.3:1	11–6.24	176
Left	♂ + ♀	13–3.48	37–9.94	2.8:1	24–6.45	372

shows the distribution of thenar/first interdigital patterns among 3088 students according to handedness, sex, and side. Note the consistent decrease in ratio of left to right among left-handers as contrasted with right-handers. This holds true for both males and females, although bimanual differences are greater among males than among females. The most striking difference is the increase of patterns on right-hands among left-handers, an increase of from slightly over 1% to almost 3.5%. Patterns on left hands show increases among left-handed males and decreases among left-handed females. No real differences are indicated in the frequencies of individuals having patterns on both hands.

The higher pattern frequencies among left-handers are due chiefly to higher frequencies of individuals having patterns on right hands only. This results in a lower bilateral asymmetry in the population of left-handers, as contrasted with the right-handers. This is in accord with the trends indicated in earlier investigations, and found in other pattern areas. The differences between right- and left-handers are highly significant, as shown in Table 6-b.

ARCHES ON MIDDLE FINGERS

Cromwell and Rife (1942) found a slightly higher frequency (1.3%) of whorls on left ring fingers of left-handers than of right-handers among those lacking whorls on the right ring finger. Although this is a trend towards lower bimanual asymmetry, the difference was statistically insignificant. Moreover, whorls occur on around 70% of the ring fingers among Caucasians, thus necessitating the collection of a tremendous number of prints to determine whether or not these differences appear to be significant. I decided, therefore, to compare the distribution of arches on middle fingers among right-handers and left-handers. Arches are actually patternless configurations having ridge counts of zero, whereas whorls with large counts are at the opposite phenotypic extreme.

Ridge counts have been estimated to possess a heritability of 90% (Holt, 1952). Genes affecting finger-tip patterns appear to affect all digits, although the incidences vary bimanually and from one finger to another. Arches occur with greatest frequencies on the left middle finger. The total frequencies of arches among North American Caucasians are from about 4% to 7%, thus rendering it feasible to test the significance of small differences with fewer numbers of individuals than would be required to run similar tests for whorls.

TABLE 3.—THE DISTRIBUTION OF ARCHES ON MIDDLE FINGERS AMONG RIGHT HANDERS AND LEFT-HANDERS

Handedness	Sex	Right Fingers Only	Left Fingers Only	Ratio Left to Right	Both	Total Number of Persons
Right.....	♂	25–2.04%	48–4.00%	1.9:1	35–2.92%	1198
Right.....	♀	27–1.78	95–6.25	3.5:1	56–3.68	1518
Right.....	♂ + ♀	52–1.91	143–5.26	2.7:1	91–3.35	2716
Left.....	♂	6–3.06	9–4.59	1.5:1	5–2.55	196
Left.....	♀	7–3.98	7–3.98	1:1	4–2.27	176
Left.....	♂ + ♀	13–3.49	16–4.30	1.2:1	9–2.41	372

The distributions of arches on middle fingers are shown in Table 3. The trends throughout parallel those recorded for the distributions of thenar/first interdigital patterns in Table 2. Both males and females show reductions in the ratios of arches on lefts only to rights only, due mostly to increases of arches on right fingers. Table 6-c shows that the increase is statistically significant, although not highly so. The lesser significance of the increase in arches is probably due to the fact that the ratio of left only to right only is smaller among the right-handers than the corresponding ratio for thenar/first interdigital patterns among right-handers. Table 6-a shows the combined distributions of thenar/first interdigital patterns and arches on middle fingers. The differences between right-handers and left-handers are highly significant. The calculated frequencies are enclosed in parentheses beneath the observed. Note that the greatest deviations between observed and calculated are for the occurrence of thenar/first interdigital palm patterns, and arches on the middle fingers of right hands only.

A further comparison was made between the finger prints of 12 families consisting entirely of right-handers, and those of 25 families of whom one or more members of each were left-handed. Almost 30% of these from families having left-handers were classed as left-handed. If one assumes that most persons heterozygous for handedness become functional right-handers because of their environments, he may assume that the gene for left-handedness occurs with a frequency of over 50% within these families. Where both parents are right-handed, each would have to be heterozygous to produce left-handed children, and where one or both are left-handed the frequencies of the gene for left-handedness would tend to be above 50%. Families consisting entirely of right-handers should have much higher frequencies of the gene for right-handedness. If the family groups are sufficiently large, we should expect to see these

TABLE 4.—THE DISTRIBUTION OF ARCHES ON MIDDLE FINGERS AMONG 25 FAMILIES OF WHICH ONE OR MORE MEMBERS OF EACH ARE LEFT-HANDED

Handedness	Sex	Arches			Total Number of Persons
		Right only	Left only	Both	
Right.....	♂	4	0	7	78
Right.....	♀	1	3	4	86
Left.....	♂	1	3	3	36
Left.....	♀	1	2	0	33
Totals.....		7–3.0%	8–3.4%	14–6.0%	233

TABLE 5.—THE DISTRIBUTION OF ARCHES ON MIDDLE FINGERS AMONG 12 FAMILIES, ALL OF WHOSE MEMBERS ARE RIGHT-HANDED

Sex	Right Only	Left Only	Both	Total Numbers Persons
♂	1	2	2	31
♀	0	3	2	32
♂ + ♀	1–1.58%	5–7.9%	4–6.63%	63

TABLE 6.—TESTS FOR SIGNIFICANCE OF DIFFERENCES

a. Distributions of arches and thenar/first interdigital patterns

	None	Right Only	Left Only	Both	Total	x^2	*df*	*p*
Right-handers	2049 (2029.8)	84 (96.8)	337 (343)	246 (246.4)	2716	15.97	3	.001 (approximately)
Left-handers	260 (279.2)	26 (13.2)	53 (47)	33 (32.6)	372			

b. Frequencies of thenar/first interdigital patterns on right only

	Right Only	Not on Right Only	Total	x^2	*df*	*p*
Right-handers	32	2684	2716	12.72	1	<.0005
Left-handers	13	359	372			

c. Frequencies of arches on middle fingers, right only

	Right Only	Not on Right Only	Total	x^2	*df*	*p*
Right-handers	52	2664	2716	4.00	1	Between <.05 and .01
Left-handers	13	359	372			

d. Comparisons of the ratios of arches on middle fingers, left-handers + families with 1 or more left-handers versus right-handers + families, all of whose members are right handed

	Right Only	Left Only	Total	x^2	*df*	*p*
Left-handers + families with left-handers	20	24	44	6.29	1	<.01 (approximately)
Right-handers + families with no left-handers	53	148	201			
Total	73	172	245			

differences reflected in the hand prints. Tables 4 and 5 show the distributions of arches within the two family groups. (Palm prints were not available for comparisons.) Note that differences are indicated, the right-handed group showing opposite asymmetry to the left-handed group. In view of the fact that the family groups are not large enough to show significant differences between them, the frequencies of arches, on one side only, within the family group were pooled with those of the student populations, as shown in Table 6-d. Note that the left-handers and the families with left-handers differ with a high degree of significance from the right-handers and families of right-handers.

THE CROMWELL SERIES

Subsequent to the analysis of the student data, a similar analysis of the distribution of arches on the middle fingers was made of the prints in the Cromwell series

TABLE 7.—THE DISTRIBUTION OF ARCHES ON MIDDLE FINGERS AMONG RIGHT-HANDERS AND LEFT-HANDERS IN THE CROMWELL SERIES

Handedness	Sex	Right Fingers Only	Left Fingers Only	Ratio	Both	Total Number of Persons
Right	♂	6–2.1%	18–6.3%	3:1	4–1.2%	284
Right	♀	7–2.4	25–8.6	3.5:1	14–4.3	288
Right	♂ + ♀	13–2.2	43–7.5	3.3:1	18–3.1	572
Left	♂	15–3.6	16–3.9	1:1	11–2.6	409
Left	♀	14–4.3	15–4.6	1:1	11–3.4	323
Left	♂ + ♀	29–3.9	31–4.2	1:1	22–3.0	732

TABLE 8.—FREQUENCIES OF ARCHES ON MIDDLE FINGER, (RIGHT ONLY) COMBINED STUDENT AND CROMWELL DATA

	Right Only	Not on Right Only	Total	x^2	*df*	*p*
Right-handers	65	3223	3288	11.56	1	<.001
Left-handers	42	1062	1104			

(Cromwell and Rife, 1942). These prints were collected from Caucasian school children in southwestern Ohio, and include those of 732 left-handers and 572 right-handers. The findings are recorded in Table 7. Note that the distributions correspond closely with those in the student population (Table 3). The incidence of arches on right hands only shows highly significant differences between left-handers and right-handers in the combined student and Cromwell series (Table 8).

DISCUSSION

The consistency and significance of the differences between right-handers and left-handers with respect to thenar/first interdigital palmar patterns and arches on middle fingers confirm earlier indications that prints of groups of left-handers manifest less bimanual asymmetry than do prints from groups of right-handers. That is to say, the bimanual variations are reduced in the total pattern frequencies of a large group of left-handers, although the number of individuals manifesting asymmetry is increased. For example, suppose prints are obtained from 100 right-handers and 100 left-handers. Six of the right-handers and also six of the left-handers have thenar/first interdigital patterns on both hands; among the right-handers 10 have these patterns on the left hand only and one has it on the right hand only; among the left handers 10 have the patterns on the left only while 4 have them on the right hand only. Among the 100 right-handers we have a total of 16 patterns on left hands and 11 on right hands, whereas among the 100 left-handers, 16 have patterns on left hands and 14 have them on right hands. The left-right pattern ratio among right-handers is 16:11, whereas it is 16:14 among the left-handers. Thus the total bimanual asymmetry is less for the 100 left-handers than for the 100 right-handers. But there are only 7 individuals among the right-handers who show bimanual asymmetry, whereas 10 of the left-handers show it. In other words, *individual* bilateral asymmetry is greater while *group* asymmetry is lesser among left-handers than among right-handers.

It should be kept in mind that these differences are of a minor nature, and are apparent only when large numbers of prints are compared. Even though the relative frequencies of thenar/first interdigital patterns and arches on middle fingers are greater among left-handers than among right-handers, the majority of people who possess either of these configurations are right-handers. According to our findings, 1 out of every 5 persons having a thenar/first interdigital pattern on the right hand only is a left-hander, and approximately 3 out of every 10 persons having an arch on his right middle finger and not on his left one, is also a left-hander. But left-handers are over 3 times as likely as right-handers to have thenar/first interdigital patterns on right hands only, and over twice as likely to have arches on the right middle finger. What may at first appear to be a discrepancy between the last two statements is due to the fact that only a small proportion of the population is left-handed.

The reason for these differences between right- and left-handers is still a matter of conjecture. Presumably the expressivities of many of the genes affecting dermatoglyphic configurations are variable, dependent upon the individuals genotype with respect to handedness. It is of interest to note that the palmar dermatoglyphics of mongoloid imbeciles deviate markedly from those of normal persons, one of their most striking features being the accentuation of normal dextral trends. Patterns occur with unusually high frequencies on the second and third interdigital areas, and with unusually low frequencies on the fourth and thenar/first interdigital areas. In at least the latter respect, the deviation from normal right-handers is in the opposite direction to that observed among left-handers.

Why do patterns occur on both hands of some persons, and on only one hand of others? Possibly those showing them on one side only are heterozygous or genotypically intermediate; that is to say they are genotypically near a threshold for the pattern expression. The embryos of left-handers may develop more symmetrically than do those of right-handers. Among the former it is more a matter of chance as to which hand a single pattern develops upon, than it is among the latter. The final solutions to these intriguing problems in human developmental genetics must await further investigation.

SUMMARY

Extensive comparisons of the finger and palm prints of right-handers with those of left-handers reveal slight but consistent and highly significant trends towards greater bilateral symmetry among groups of left-handers.

These trends are most readily apparent through comparisons of the distributions of patterns on the thenar/first interdigital area of the palm and arches on middle fingers. These associations between dermatoglyphics and handedness, as well as those indicating linkage, demonstrate conclusively that handedness cannot depend solely upon postnatal circumstances. The phenotypic expression of handedness is dependent upon both heredity and environment.

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The Use of Multi-Allele Genetic Characters in the Diagnosis of Twin Zygosity

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ALTHOUGH THE DIAGNOSIS of twin zygosity by means of genetic systems is of considerable importance in human genetics, there is still some confusion concerning the appropriate procedures to be employed when a particular pair of twins is being considered. Most of the contributions to the subject, notably those of Wiener and Leff (1940), Fisher (1951), Cotterman (1951), Wiener (1952), and Race and Sanger (1954), have dealt primarily with the efficiency of certain genetic systems in recognizing dizygosity in populations of twins. Some authors, however, have used the methods appropriate to this problem in diagnosing the zygosity of particular twin pairs.

In diagnosing zygosity by means of genetic phenotypes attention is given to whether the twins are concordant or discordant with respect to the genetic loci involved. Although discordant sets are regarded as almost certainly dizygous, much less certainty is involved in classifying concordant pairs as monozygous. It is therefore desirable to calculate the probability of monozygosity for twins who are concordant. This probability can be calculated either with or without reference to the actual phenotypes which the concordance involves. When the zygosity of a particular set of twins is under consideration it is appropriate to calculate the probability of monozygosity *with* reference to the actual phenotypes involved, whereas when the probability of monozygosity is desired for a random pair of twins or when the adequacy for diagnostic purposes of a proposed series of genetic phenotypes is in question it is appropriate to calculate the probability of total concordance *without* reference to the actual phenotypes. Although this distinction was made by Fisher (1951) it has been neglected by subsequent authors.

Another source of error has been those cases in which the genotype of an individual is not known from his phenotype. The literature has usually considered only the most probable genotype in such cases. Because of current interest in these matters the authors feel that a general discussion of the diagnosis of twin zygosity by genetic phenotypes is appropriate.

PROPORTION OF CONCORDANT PAIRS WHICH ARE MONOZYGOUS

If we let

$P(M)$ = the *a priori* probability of a pair of twins being monozygous,
$P(D)$ = the *a priori* probability of a pair of twins being dizygous,
$P(M.C)$ = the probability of a pair of twins being both monozygous and concordant in all of the f independent loci considered,
$P(D.C)$ = the probability of a pair of twins being both dizygous and concordant in all of the f independent loci considered,

Received January 24, 1955.

$P(C \mid M)$ = the probability of a pair of monozygous twins being concordant in all f independent loci,

$P(C_i \mid D)$ = the probability of a pair of dizygous twins being concordant in the ith locus,

then $P(C \mid D)$, the probability of a pair of dizygous twins being concordant in all of the f independent loci considered, is $\prod_{i=1}^{f} P(C_i \mid D)$; and $P(M \mid C)$, the probability of a pair of twins who are concordant in all f independent loci being monozygous, is (Race and Sanger, 1954)

$$P(M \mid C) = \frac{P(M.C)}{P(M.C) + P(D.C)}. \quad \text{Since} \tag{1}$$

$$P(M.C) = P(M)P(C \mid M) = P(M) \text{ and} \tag{2}$$

$$P(D.C) = P(D)P(C \mid D), \text{ then} \tag{3}$$

$$P(M \mid C) = \frac{P(M)}{P(M) + P(D)P(C \mid D)}. \tag{4}$$

In the preceding discussion the nature of the concordance has not been specified. In formulas (1), (3), and (4) $P(C_i \mid D)$ will be denoted by $P(C_i \mid D)_s$ when applied to a particular phenotype and by $P(C_i \mid D)_r$ when it represents the probability of concordance for a random phenotype, with corresponding meanings for $P(C \mid D)_s$ and $P(C \mid D)_r$. Likewise the probability of monozygosity for concordant twins will be designated by $P(M \mid C)_s$ and $P(M \mid C)_r$ respectively, depending upon whether a particular or a random pair of twins is under consideration.

PROBABILITY OF CONCORDANCE FOR DIZYGOUS TWINS OF SPECIFIED PHENOTYPE

When the probability of monozygosity is desired for a particular pair of concordant twins the actual phenotypes of the twins should be utilized. That is, $P(C_i \mid D)_s$ is equivalent to $P[(\phi_{Ti} \mid \phi_{Ti}) \mid D]$, where ϕ_{Ti} denotes the phenotype of the twins at the ith locus. Correspondingly,

$$P(C \mid D)_s = \prod_{i=1}^{f} P[(\phi_{Ti} \mid \phi_{Ti}) \mid D]. \tag{5}$$

For brevity $P[(\phi_{Ti} \mid \phi_{Ti}) \mid D]$ will hereafter be represented by $P(\phi_T \mid \phi_T)$, it being understood that a particular locus is involved.

The value of $P(\phi_T \mid \phi_T)$ is a function of the parental genotypes as well as of the particular phenotype represented by ϕ_T. The information about parental genotypes, however, varies with the situation. They may be completely known, or it may be that the only information about them is that which can be deduced from their offspring and a knowledge of the gene frequencies of the population involved.

Consider a mating about whose genotypes a certain amount of information is available and which has produced, in addition to the twins whose zygosity is in question, s full sibling offspring whose phenotypes at a given locus are $\phi_1, \phi_2, \cdots \phi_s$. Let n

denote the number of possible genotypic mating combinations which, on the basis of the available information, the parents might be. The probability of the parental genotypes being any specified combination m_k of the n possible combinations is

$$(6) \qquad P(m_k \mid \phi_1, \phi_2, \cdots \phi_s) = \frac{P(m_k) \prod_{h=1}^{s} P(\phi_h \mid m_k)}{\sum_{j=1}^{n} \left[P(m_j) \prod_{h=1}^{s} P(\phi_h \mid m_j) \right]}$$

where $P(m_j)$ is the probability, based solely on gene frequencies, of parental mating combination m_j and $P(\phi_h \mid m_j)$ is the probability of obtaining from mating combination m_j an offspring of phenotype ϕ_h. The probability of another full sibling being of any specified phenotype ϕ_X is

$$(7) \qquad P(\phi_X \mid \phi_1, \phi_2, \cdots \phi_s) = \frac{\sum_{j=1}^{n} \left[P(m_j) \prod_{h=1}^{s} P(\phi_h \mid m_j) P(\phi_X \mid m_j) \right]}{\sum_{j=1}^{n} \left[P(m_j) \prod_{h=1}^{s} P(\phi_h \mid m_j) \right]}.$$

The special case with which we are here concerned is the probability of a dizygous twin of an offspring of phenotype ϕ_T also being of phenotype ϕ_T. This probability is

$$(8) \qquad P(\phi_T \mid \phi_T) = \frac{\sum_{j=1}^{n} \left[P(m_j) \left[\prod_{h=1}^{s} P(\phi_h \mid m_j) \right] [P(\phi_T \mid m_j)]^2 \right]}{\sum_{j=1}^{n} \left[P(m_j) \left[\prod_{h=1}^{s} P(\phi_h \mid m_j) \right] P(\phi_T \mid m_j) \right]}.$$

EXAMPLE UTILIZING FORMULA (8)

As an example of the use of formula (8) we choose the case of a woman of blood types A_1B and M producing a set of concordant male twins of blood types A_1 and M.

TABLE I.—PROBABILITIES OF OFFSPRING PHENOTYPES FROM VARIOUS MATINGS, BASED ON *ABO* LOCUS

(1) Mating	(2)* Probability of Male Genotype		(3) Probability of Two Full Non-Twin Sibs of Type A_1	(4) Probability of Obtaining a Dizygous Twin of Type A_1	(5) (2) × (3) × (4)	(6) (2) × (3) × (4)²
$A_1A_1 \times A_1B$	a_1^2	.0437	1/4	1/2	.00546	.00273
$A_1A_2 \times A_1B$	$2a_1a_2$	.0291	1/4	1/2	.00364	.00182
$A_1B \times A_1B$	$2a_1b$	.0256	1/16	1/4	.00040	.00010
$A_1O \times A_1B$	$2a_1o$	.2760	1/4	1/2	.03450	.01725
$A_2A_2 \times A_1B$	a_2^2	.0048	1/4	1/2	.00060	.00030
$A_2B \times A_1B$	$2a_2b$	.0085	1/16	1/4	.00013	.00003
$A_2O \times A_1B$	$2a_2o$	.0919	1/4	1/2	.01149	.00574
$BB \times A_1B$	b^2	.0037	0	0	.00000	.00000
$BO \times A_1B$	$2bo$	.0808	1/16	1/4	.00126	.00032
$OO \times A_1B$	o^2	.4359	1/4	1/2	.05449	.02724

* The entries in column (2) are calculated from the gene frequencies given in Table V.

TABLE II.—PROBABILITIES OF OFFSPRING PHENOTYPES FROM VARIOUS MATINGS BASED ON MN LOCUS

(1) Mating	(2)* Probability of Male Genotype		(3) Probability of Non-Twin of Type MM	(4) Probability of Non-Twin of Type MN	(5) Probability of Twin of Type MM	(6) (2) × (3) × (4) × (5)	(7) (2) × (3) × (4) × (5)^2
$MM \times MM$	m^2	.284622	1	0	1	0	0
$MN \times MM$	$2mn$	.497756	1/2	1/2	1/2	.06222	.03111
$NN \times MM$	n^2	.217622	0	1	0	0	0

* The gene frequencies used in column (2) are given in Table V.

Two other single born siblings of the twins are of blood types A_1MN and A_1M. The father is unavailable for testing. Beginning with the ABO locus we enumerate the ten possible matings with their associated probabilities as shown in Table I.

Applying formula (8),

$$P(A_1 \mid A_1) = \frac{\sum \text{column (6)}}{\sum \text{column (5)}} = \frac{.0558}{.1120} = .498.$$

In like manner the possible matings with respect to the MN system are enumerated in Table II. In this case, however, the mating is completely specified, so that

$$P(MM \mid MM) = \frac{\sum \text{column (7)}}{\sum \text{column (6)}} = \tfrac{1}{2}.$$

Making use of the fact that the twins are of the same sex, we have

$$P(\sigma^{\prime} \mid \sigma^{\prime}) = 1/2.$$

$$\text{Now } P(C \mid D)_s = P(A_1 \mid A_1)P(MM \mid MM)P(\sigma^{\prime} \mid \sigma^{\prime}) = .1245.$$

Using the values .66 and .34 for $P(D)$ and $P(M)$ respectively (Strandskov and Edelen, 1946) we finally obtain from formula (4)

$$P(M \mid C)_s = .81.$$

PROBABILITY OF MONOZYGOSITY BASED ONLY ON TWIN PHENOTYPES AND POPULATION GENE FREQUENCIES

The probability, $P(\phi_T \mid \phi_T)$, for the common situation in which the only information about the parents is derived from the twin phenotype and population gene frequencies is designated by $P(\phi_T \mid \phi_T)_z$. It has the value

$$P(\phi_T \mid \phi_T)_z = \frac{\sum_{j=1}^{n} [P(m_j)[P(\phi_T \mid m_j)]^2]}{\sum_{j=1}^{n} [P(m_j)P(\phi_T \mid m_j)]}. \tag{9}$$

Formulas (8) and (9) are general in the sense that they apply equally to multiple alleles, various dominance relations, sex-linkage, etc., provided the genetic mechanism is known.

Although $P(\phi_T \mid \phi_T)_Z$ can always be calculated by means of (9), it can also be obtained in a more direct and simple manner, as is shown in the following section.

RECOMMENDED PROCEDURE FOR CALCULATION OF $P(\phi_T \mid \phi_T)_Z$

Consider an offspring whose genotype, at a given locus, is $(G_\alpha G_\beta)$. The four gene positions of the parents may be represented as 1, 2, 3, and 4, the positions carrying G_α and G_β being arbitrarily numbered 2 and 4 respectively and the other positions being arbitrarily numbered 1 and 3. Four combinations of these positions are possible in the progeny, namely: 1,3; 1,4; 2,3; 2,4; each with the probability 1/4. The probabilities from each positional combination of obtaining another offspring of genotype $(G_\alpha G_\beta)$ are

$$1,3 = 2\alpha\beta/4$$

$$1,4 = \alpha/4$$

$$2,3 = \beta/4$$

$$2,4 = 1/4$$

so that the total probability

$$P[(G_\alpha G_\beta) \mid (G_\alpha G_\beta)]_Z = \frac{1 + \alpha + \beta + 2\alpha\beta}{4}, \tag{10}$$

where α and β represent the population frequencies of G_α and G_β respectively. In applying this method it is not necessary that the alleles to the right of the | be the same as those on the left. The value of any $P[(G_\alpha G_\beta) \mid (G_\gamma G_\delta)]_Z$ can be obtained in this manner, the various types being presented in formulas (14) to (21).

In many cases an individual's genotype is not known from his phenotype. This is true for individuals possessing any dominant allele as well as for certain of the Rh blood types, where several totally different genotypes may be represented by the same phenotype. Consider, for example, the simple but easily generalized situation in which the possible genotypes represented by a given individual are $\phi_T = (G_\alpha G_\beta)$ v $(G_\gamma G_\delta)$, where any of the G_α, G_β, G_γ, and G_δ may or may not be different from one another.[1] It is evident that

$$P[(G_\alpha G_\beta) \text{ v } (G_\gamma G_\delta) \mid (G_\alpha G_\beta)]_Z = P[(G_\alpha G_\beta) \mid (G_\alpha G_\beta)]_Z + P[(G_\gamma G_\delta) \mid (G_\alpha G_\beta)]_Z \tag{11}$$

and that

$$P[(G_\alpha G_\beta) \text{ v } (G_\gamma G_\delta) \mid (G_\gamma G_\delta)]_Z = P[(G_\alpha G_\beta) \mid (G_\gamma G_\delta)]_Z + P[(G_\gamma G_\delta) \mid (G_\gamma G_\delta)]_Z . \tag{12}$$

By taking the weighted mean of (11) and (12) we obtain

$$P(\phi_T \mid \phi_T)_Z = \frac{X_{\alpha\beta}\,\alpha\beta P[(G_\alpha G_\beta) \text{ v } (G_\gamma \hat{G_\delta}) \mid (G_\alpha G_\beta)]_Z + X_{\gamma\delta}\,\gamma\delta P[(G_\alpha G_\beta) \text{ v } (G_\gamma G_\delta) \mid (G_\gamma G_\delta)]_Z}{X_{\alpha\beta}\,\alpha\beta + X_{\gamma\delta}\,\gamma\delta}, \tag{13}$$

where $X_{\alpha\beta} = 1$ if $\alpha \equiv \beta$ and 2 if $\alpha \not\equiv \beta$, and

$X_{\gamma\delta} = 1$ if $\gamma \equiv \delta$ and 2 if $\gamma \not\equiv \delta$.

[1] The symbol v is to be read and/or.

By appropriate extension of this method $P(\phi_T \mid \phi_T)_z$ can be obtained for any phenotype however complex. The elementary probabilities required, obtainable by the method of the previous paragraph, are:

$$P(G_\alpha G_\alpha \mid G_\alpha G_\alpha)_z = \frac{1 + 2\alpha + \alpha^2}{4} \tag{14}$$

$$P(G_\alpha G_\alpha \mid G_\alpha G_\beta)_z = \frac{\alpha + \alpha^2}{4} \tag{15}$$

$$P(G_\alpha G_\alpha \mid G_\beta G_\beta)_z = \frac{\alpha^2}{4} \tag{16}$$

$$P(G_\alpha G_\alpha \mid G_\beta G_\gamma)_z = \frac{\alpha^2}{4} \tag{17}$$

$$P(G_\alpha G_\beta \mid G_\alpha G_\alpha)_z = \frac{2\beta + 2\alpha\beta}{4} \tag{18}$$

$$P(G_\alpha G_\beta \mid G_\alpha G_\beta)_z = \frac{1 + \alpha + \beta + 2\alpha\beta}{4} \tag{19}$$

$$P(G_\alpha G_\beta \mid G_\alpha G_\gamma)_z = \frac{\beta + 2\alpha\beta}{4} \tag{20}$$

$$P(G_\alpha G_\beta \mid G_\gamma G_\delta)_z = \frac{2\alpha\beta}{4} \tag{21}$$

The symbols α, β, γ, and δ above represent population frequencies of genes G_α, G_β, G_γ, and G_δ, respectively.

SOME PARTICULAR CASES OF $P(\phi_T \mid \phi_T)_z$

Most genetic systems can be represented by a few patterns of dominant/recessive relationships. The following special cases are often applicable.

I. *Homozygous genotype.* In this case the phenotype is sufficient to establish the homozygous nature of the genotype, such as for recessive or non-dominant alleles. Examples are blood types M, N, O, kk, Fy^a-, rr, $R'R'$, $R''R''$, R^yR^y, or the non-secretor type. *Throughout this paper the results relating to Rh types are based on the antisera C, D, E, c, and e.*

$$P(G_\alpha G_\alpha \mid G_\alpha G_\alpha)_z = \frac{(1 + \alpha)^2}{4}, \tag{22}$$

where α on the right-hand side of the equation represents the population frequency of G_α.

II. *Heterozygous non-dominant genotype.* This applies to those cases in which the phenotype reveals the specific alleles present in the genotype. Examples are blood types MN, A_1B, A_2B, $R'r$, $R''r$, $R'R^y$, and $R''R^y$.

$$P(G_\alpha G_\beta \mid G_\alpha G_\beta)_z = \frac{1 + \alpha + \beta + 2\alpha\beta}{4}, \tag{23}$$

where α and β on the right represent population frequencies for genes G_α and G_β.

TABLE III.—APPROPRIATE GENE FREQUENCIES ASSOCIATED WITH EACH PHENOTYPE

Phenotype*	α	β
A_1	A_1	$A_2 + O$
A_2	A_2	O
B	B	O
R_1R_1	R_1	R'
R_2R_2	R_2	R''
R_0r	R_0	r
R^zR^z	R^z	R^y

* The Rh phenotypes are represented by their most probable genotypes.

III. *"Complete" dominance.* This group includes those phenotypes in which an allele is present dominant to all other alleles. Examples are the blood types K^+, $Fy^a(+)$, and the presence of the secretor factor.

$$P(G_\alpha \mid G_\alpha)_Z = \frac{\alpha^3 - 6\alpha^2 + 5\alpha + 4}{4(2 - \alpha)}, \tag{24}$$

where α, on the right, is the population frequency of gene G_α.

IV. *Limited dominance.* This phenotype is produced by an allele G_α either in the homozygous state or in combination with alleles G_β to which G_α is dominant. There exist other alleles to which G_α is recessive or non-dominant. The gene frequency represented by β is the sum of all such recessive alleles G_β since no distinction between them need be made. Examples are the phenotypes A_1, A_2, B, "R_1R_1", "R_2R_2", "R_0r", and "R^zR^z".

$$P(G_\alpha \mid G_\alpha)_Z = \frac{\alpha(\alpha + 2\beta)^2 + (1 + 2\alpha)(\alpha + 2\beta) + 2\beta(\alpha + \beta)}{4(\alpha + 2\beta)}. \tag{25}$$

The gene frequencies which are represented by α and β are indicated in Table III for each phenotype. The Rh types are expressed in terms of the most probable genotype.

V. *Complex heterozygous phenotype.* Three of the most common Rh phenotypes, designated by their most probable genotypes "R_1R_2", "R_1r", and "R_2r", along with the rare "R_1R^z" and "R_2R^z", are each composed of a group of indistinguishable heterozygous genotypes. Four of these Rh types show the same pattern of genotypic constitution and the fifth shows this pattern repeated twice. For purposes of exposi-

TABLE IV.—APPROPRIATE GENE FREQUENCIES FOR FORMULA (26)

Most Probable Genotype	α	β	γ	δ	ϵ	ζ	η	θ
R_1R_2	R_1	R_2	R''	R'	R_0	R^z	R^y	r
R_1r	R_1	R_0	r	R'	—	—	—	—
R_2r	R_2	R_0	r	R''	—	—	—	—
R_1R^z	R_1	R^z	R^y	R'	—	—	—	—
R_2R^z	R_2	R^z	R^y	R''	—	—	—	—

Where dashes appear, the value zero should be substituted in the formula.

tion, these five have been represented by one formula, with the appropriate substitutions indicated in Table IV.

$$(26)\quad P(G_R \mid G_R)_Z = \frac{2R^2 + R + \alpha(\beta+\gamma)^2 + \beta(\alpha+\delta)^2 + \beta^2\delta + \alpha^2\gamma + \epsilon(\zeta+\eta)^2 + \zeta(\epsilon+\theta)^2 + \zeta^2\theta + \epsilon^2\eta}{4R}$$

where $R = \alpha\beta + \alpha\gamma + \beta\delta + \epsilon\zeta + \epsilon\eta + \zeta\theta$.

TOTAL PROBABILITY OF CONCORDANCE AT A LOCUS

It sometimes happens, as for example in assessing the efficiency of a given locus in distinguishing dizygous from monozygous twins, that one wishes to estimate the total probability of concordance in all phenotypes at that locus. Although the probability appropriate to this problem, $P(C_i \mid D)_r$, is well known in the literature, it can be more readily calculated by taking the mean of the various $P(\phi_k \mid \phi_k)_Z$ weighted according to the population frequency of the phenotypes ϕ_k than by traditional methods involving the enumeration of matings. Thus

$$(27)\quad P(C_i \mid D)_r = \sum_{k=1}^{n} [P(\phi_k) P(\phi_k \mid \phi_k)_Z],$$

where n is the number of different phenotypes possible at that locus and $P(\phi_k)$ is the population frequency of phenotype ϕ_k. The numerical values of $P(C_i \mid D)_r$ for a number of human blood groups are given in Table V.

TABLE V.—NUMERICAL VALUES OF $P(C_i \mid D)_r$, THE PROBABILITY OF A RANDOM PAIR OF DIZYGOUS TWINS BEING CONCORDANT AT THE iTH LOCUS, FOR A NUMBER OF HUMAN BLOOD GROUPS

Locus	Population	Reference	Gene Frequencies	$P(C_i \mid D)_r$
ABO	Southern England	Race and Sanger, p. 24	A_1 .2090 A_2 .0696 B .0612 O .6602	.6234
MN	American Whites	Mourant, p. 357	M .5335 N .4665	.5952
Rh	American Whites	Mourant, p. 395	R_0 .0260 R_1 .4165 R_2 .1369 r .4109 R' .0045 R'' .0036 R^y .0015 R^z .0000	.4934
Kell	Minnesota Whites	Mourant, p. 404	K .0566 k .9434	.9007
Duffy	Minnesota Whites	Mourant, p. 409	Fy^a .4344 Fy^b .5656	.7523

The combined efficiency of f independent loci in distinguishing monozygous from dizygous twins can be expressed by

$$P(C \mid D)_r = \prod_{i=1}^{f} P(C_i \mid D)_r .$$

The probability of monozygosity for a random pair of twins who are concordant in all f loci, $P(M \mid C)_r$, is obtained by substituting $P(C \mid D)_r$ in (4). Since $P(M \mid C)_r$ is the harmonic mean of the various $P(M \mid C)_s$ weighted according to their population frequencies it can also be obtained by formula (28) below.

$$P(M \mid C)_r = \frac{\sum_{s=1}^{n} P(\phi_s)}{\sum_{s=1}^{n} \frac{P(\phi_s)}{P(M \mid C)_s}}, \tag{28}$$

where ϕ_s represents a specific phenotype in all f loci and n is the total number of phenotypes considered. If all of the possible phenotypes are considered

$$\sum_{s=1}^{n} P(\phi_s) = 1.$$

For most purposes $P(M \mid C)_r$ can be more readily calculated by substitution of $P(C \mid D)_r$ in (4) than by (28).

SUMMARY

The estimation of the probability of monozygosity for twins by means of genetic systems can be done in two ways, depending on whether a specific phenotype is in question or whether a random phenotype is in question. Procedures for computing the probability of monozygosity for specific phenotypes have been presented. These procedures are applicable to even the most complex phenotypes, provided the genetic mechanisms are understood. Whatever information is available from the parents and siblings can also be introduced to increase the accuracy of the results. A shortened procedure is presented for computing the probability of monozygosity for a random set of concordant twins, and the efficiency of some of the blood group systems has been calculated by this method.

Acknowledgements

This study has been supported in part by a grant from McGregor Fund of Detroit. We are indebted to Kathryn Hanchon for performing the calculations.

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Primordial Dwarfism and Ectopia Lentis

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IN THE COURSE of a study of a generalized heritable disorder of connective tissue, Marfan's syndrome, in which ectopia lentis is a frequent manifestation, attention was drawn to a family in which the ocular anomaly occurs in association with primordial (ateliotic or proportionate) dwarfism. Further study of the family revealed that the association of these two traits in some members of one sibship is a coincidence and not a true hereditary syndrome. Furthermore, in this family each of these traits demonstrates what appears to be a recessive pattern of inheritance. Presented below is the evidence on which these conclusions are based.

Although there are several genetic varieties of ectopia lentis, the cases of this anomaly are ophthalmologically indistinguishable. In all of them the primary defect appears to be one of the suspensory ligaments of the lens, resulting in physical weakness. Because the inferior ligaments are most likely to be defective, the usual displacement of the lens is upward. In at least two of the genetic types there are generalized evidences of constitutional abnormality. 1) Marfan's syndrome (McKusick, 1955) comprises also dolichostenomelia (long, thin extremities) and weakness of the media of the aorta. It is inherited as a Mendelian dominant. 2) The Weil-Marchesani syndrome (for photographs see McKusick, 1955) is characterized by brachymorphism (short stature but not dwarfism, brachycephaly, short and broad hands and digits). It likewise usually manifests a dominant pattern of inheritance. In a third group, which is possibly not fundamentally homogeneous, ectopia lentis occurs as an isolated anomaly and behaves as a recessive trait. Of all cases of hereditary ectopia lentis approximately 70%, 15%, and 15%, respectively, fall into these three groups.

Primordial dwarfism is not to be confused with achondroplastic dwarfism or the several types of dwarfism resulting from pathologic conditions of the skeleton, acquired and inherited. Characteristically the primordial dwarf is of miniature construction with approximately normal anthropometric proportions. Primordial dwarfism has been considered by most (Rischbieth and Barrington, 1912) to be a recessive trait. Hogben (1932) suggested an alternative mode of inheritance, namely one involving the interaction of two independent (i.e., on different chromosomes) but complementary genes, the presence of neither of which is manifested unless the other is present. Examination of members of kinships in which primordial dwarfism occurs (e.g., Jacobsen, 1891) suggests that heterozygosity may be expressed in the form of an intermediate degree of stunting.

THE PEDIGREE

The Negro kinship displayed in Figure 1 had its origin in a rural, tidewater section of eastern Virginia. The surnames of the three principal lines in this geneology are

Received March 14, 1955.

frequently occurring ones among Negroes, having been the names of prominent white families in that area. No instances of either dwarfism or ectopia lentis were discovered in the kinship other than those in the sibship of the fourth generation (IV 13–22). Four individuals in this sibship of nine were dwarfed and four demonstrated ectopia lentis. The two anomalies coincided in two individuals. Two males and two females showed ectopia lentis; one male and three females dwarfism.

Consanguinity was a conspicuous feature. The parents of the affected sibship were at least first cousins. By some reports illegitimate paternity of individual II 4 would give the parents of the affected sibship a degree of consanguinity intermediate between that of simple first cousins and that of double first cousins. This alternative pedigree is sketched in the inset of Figure 1.

All members of this kinship were of average height, about 5′4″ for the most part. The father of the affected sibship was about 61 inches tall and of slight build, weighing only about 98 pounds. He was not available for examination, having been shot fatally in 1942. He had no known visual abnormality. The mother of the affected sibship was 48 years old at the time of examination in 1953, was 63 and ½ inches tall

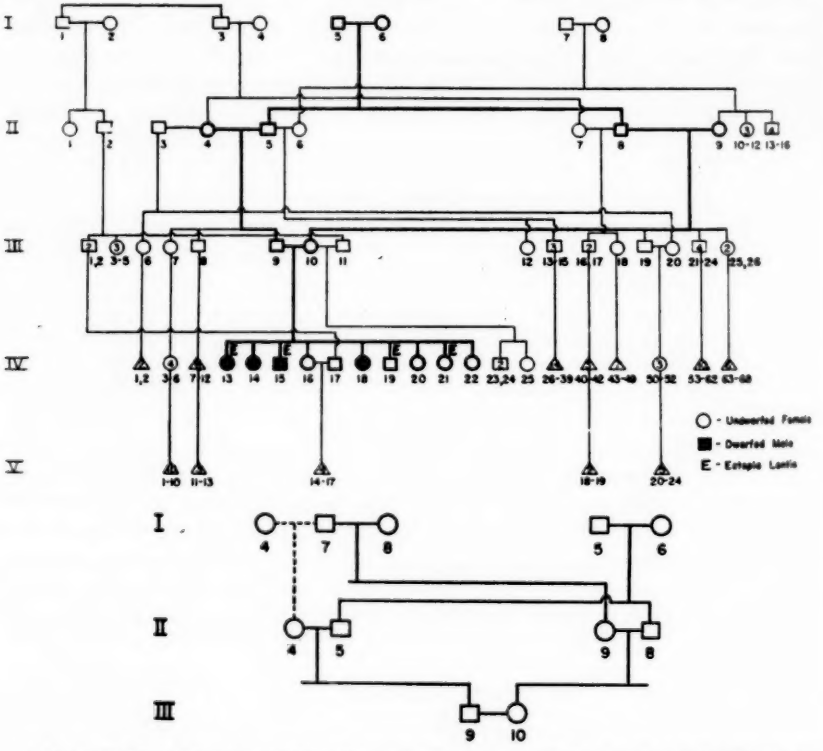
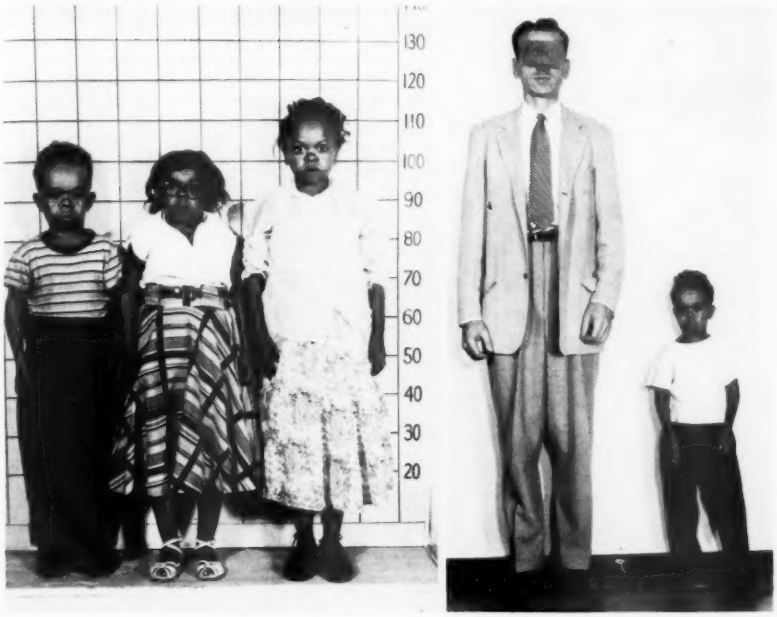


FIG. 1. Pedigree. See text. The inset shows an alternative possibility for the relationships in the first two generations.



a b

FIG. 2a. Left to right, individuals IV 15, IV 13, and IV 18. The first two also have ectopia lentis with complicating glaucoma. Not photographed is individual IV 14, who next to IV 15 is the shortest member of the family.

2b. These individuals, of approximately the same age, are six feet and 39 inches tall, respectively.

and weighed 220 pounds. The facial configuration of her dwarfed children resembled hers closely. Specifically, her nose showed a very wide and depressed bridge and pronounced tip-tilt. The second husband of this individual (III 11) is approximately 66 inches tall. His eyes are normal and he is otherwise well.

All members of the affected sibship are of average intelligence. One of the dwarfs (IV 18) completed high school. All four dwarfs weighed five to six pounds at birth (approximately the same as their undwarfed siblings) and were not considered particularly small until the age of 12 months to three years. Talking and walking developed at a normal age. Sexual function is apparently normal in all, although catamenia was tardy and pregnancy has not occurred. The voice in all four dwarfs is rather shrill and piping, that in the male dwarf being deeper, however.

Bilateral ectopia lentis has led to near-blindness from glaucoma in patient IV 13. In patient IV 15 glaucoma has rendered one eye sightless, but the other has been saved by lens extraction after a bout of acute glaucoma. In a third patient (IV 19), one dislocated lens is symmetrically incarcerated in the pupil and presents a most dramatic appearance to direct inspection. In the fourth sib with ectopia lentis (IV 21), iritis of an irritative type has occurred, but tension to digital examination was

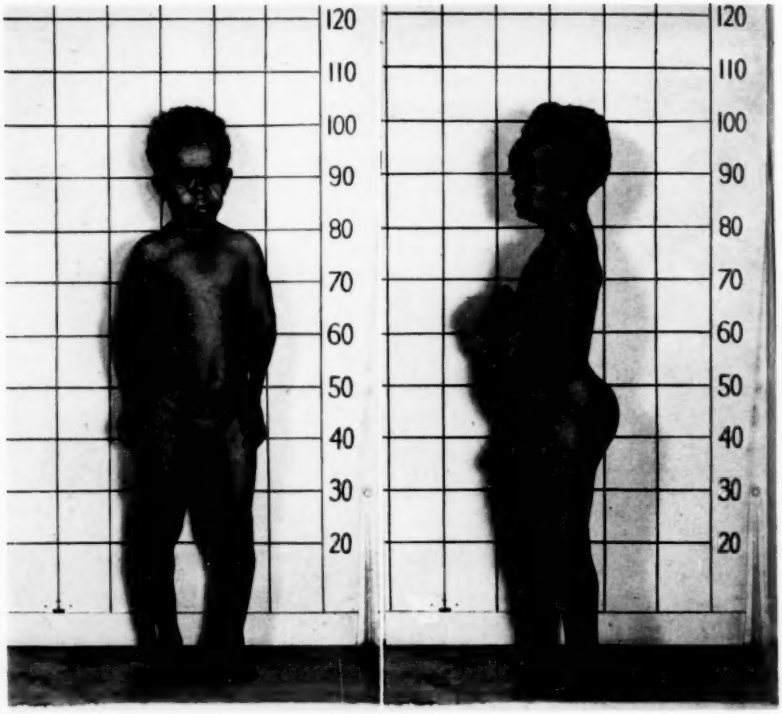


FIG. 3. Frontal and lateral views in the nude of IV 15.

normal at the time she was seen. All four patients with ectopia lentis have severe myopia, whereas the five other sibs are essentially emmetropic.

Detailed description of the affected sibship (IV 13–25) follows:

IV 13. Eleanora, born in 1920, has both anomalies. She is 40 inches tall and weighs 49 pounds. Catamenia occurred at the age of 20 years and menses have been normal since. High grade myopia is present. In the left eye there is an obvious dislocation of a cataractous and spherophakic lens into the anterior chamber. Both irides are markedly atrophic. The right lens was removed and iridectomy performed at another hospital in 1952. Both eyes are glaucomatous and vision is severely impaired. The patient refuses further ophthalmological treatment. Radiologic survey of the entire skeleton reveals a complete epiphyseal closure.

IV 14. Irene, born in 1921, is now 39½ inches tall and weighs 50 pounds. She has normal eyes. Catamenia occurred at the age of 18 years and menses have been normal since.

IV 15. Mitchell*, born in 1924, has ectopia lentis and is only 39 inches tall with a

* I am indebted to Dr. Melvin Grumbach, Dr. Lawson Wilkins, and Dr. Frank B. Walsh for examination of this patient.

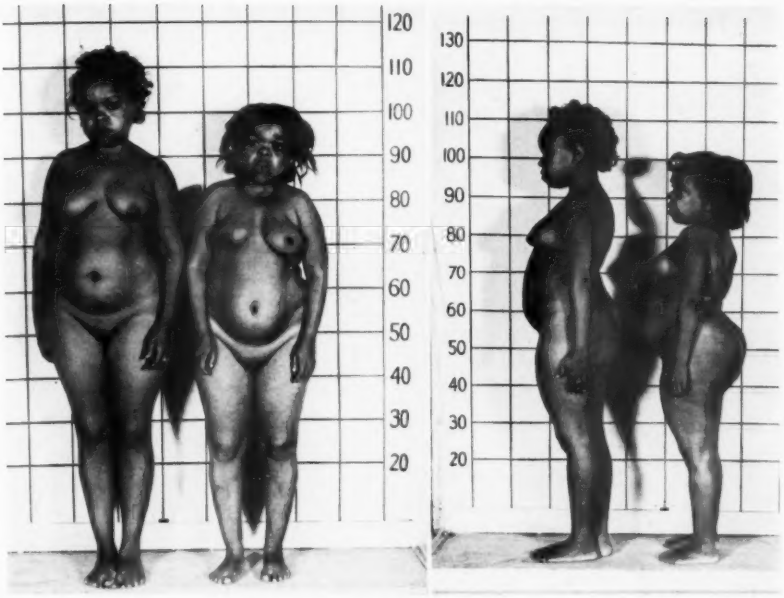


FIG. 4. Frontal and lateral views of IV 18 and IV 13.

weight of 34.5 pounds. His short stature was first noted after the age of one year. He completed grade school and worked for about ten years in travelling shows and as a night-club entertainer. Pubic hair appeared between the ages of 12 and 14 years. He began to have erections at age 14 and masturbated at the age of 18 years. He has an erection about once a week and has had ostensibly normal sexual relations. He wore glasses in school but the first serious visual difficulty, glaucoma, began in the left eye in 1948. Complete loss of vision in this eye occurred during the next few years. In 1952 vision in the right eye began to fail. The patient uses tobacco and alcohol in liberal amounts, and his tolerance for both is apparently high.

Physical examination revealed a normally proportioned, strikingly dwarfed man. Micrognathia, depressed nasal bridge, and moderate lumbar lordosis were present. His facial expression was mature. His height corresponds to that of a $3\frac{3}{4}$ year old child. The ratio of upper and lower segment measurements was 1.12, a value in excess of the normal adult value of 0.98. The hands and feet were very small but normally proportioned. The dentition was fully adult but there was malalignment and malocclusion. The testis measured 3.5 x 2.5 cm. and the penis 9 x 2.2 cm. There was abundant curly pubic hair with a female escutcheon. There was no hair elsewhere on the body and no facial hair except for a sparse amount over the upper lip. The scalp hair showed normal male recession in the frontal and temporal areas.

The findings of general physical examination were within normal limits except for borderline enlargement of the heart, moderate accentuation of the second aortic sound, and a blood pressure of 145/105 mm. Hg when measured with a pediatric cuff.

FIG. 5. Other than the proportionate dwarfism, the only definite abnormality revealed by x-ray study of the entire skeleton in individuals IV 15, IV 13, and IV 18 was delayed fusion of the epiphyses at the iliac crests in individual IV 15 who is 29 years old.

X-rays of the skeleton revealed that all the epiphyses were fused except for those of the iliac crests, which, however, were in the process of fusing (Fig. 5). The sella turcica was of normal size in proportion to that of the skull. The heart was enlarged mainly in its left ventricular salient. The assay of urinary FSH revealed something more than 6.6 m.u/24 hrs. The urinary 17-ketosteroid estimation gave a result of 2.0 mg/24 hrs. Corrected for body surface area (about 0.89 m^2) the latter value is not strikingly abnormal. The EKG was normal.

(The cardiomegaly in this patient may be related, at least in part, to his mild hypertension. It was speculated that in part it might represent an "athlete's heart"

of sorts resulting from the patient's having continually to exert himself in keeping up with his long-legged companions!)

Ophthalmologic examination revealed absolute glaucoma in the left eye and acute glaucoma in the right. The left eye had no light perception. There was bilateral ectopia lentis and iridodonesis (tremor of the iris from lack of its usual support posteriorly). Dr. Chas. Tillett performed an intra-capsular extraction of the right lens without loss of vitreous or other complications. Since then the tension has remained normal in this eye, and vision with spectacles is 20/20.

IV 16. Inez, born in 1928, has normal eyes and is 61 inches tall. She has four children of ages 4 months to six years. These are all normal in stature and have normal eyes.

IV 18. Mazey, born in 1931, is 43¾ inches tall and weighs 50 pounds. She has a much depressed nasal septum and a baby face. The eyes are normal. Slit lamp examination with the pupils widely dilated revealed no abnormality. Menstruation, which had its onset at the age of 16 years, is entirely normal. She graduated from high school in 1950. Complete radiologic survey of the skeleton revealed essentially no delay of epiphyseal union.

IV 19. Ozel, born in 1932, is 61 inches tall and weighs 115 pounds. He has ectopia lentis as an isolated anomaly. He is severely myopic. When seen at home in 1953 the right pupil was normal in size and reacted normally. The left pupil was widely dilated and the iris did not react, being held open by the lens which appeared to be of normal size but was displaced from its normal position and incarcerated in the pupil. The iris was clamped around the equator of the lens like a tight cuff.

IV 20. Frances, born in 1934, is normal in every respect. She is 61 inches tall and weighs 110 pounds.

IV 21. Marie, born in 1937, is 60 inches tall. She is severely mypoic. She has a history of inflammation in the eyes. Definite iridodonesis is present, especially on the left. The irides are atrophic and show white areas which appear to be scars. However, they react normally to light and with accommodation.

IV 22. Catharine, born in 1939, is normal in every respect. She is 60 inches tall.

Philip (IV 23), born in 1944, Harold (IV 24), born in 1946, and Cora Lee (IV 25), born in 1947 have normal eyes and are of normal stature for their ages.

COMMENTS

Genetic considerations

From examination of the pedigree in Figure 1 it seems most likely that in this family ectopia lentis and primordial dwarfism are inherited independently, that is, are due to mutant genes on different chromosomes. The high degree of consanguinity in the parents, who were themselves unaffected to the best of our knowledge, suggests a recessive mode of inheritance. The alternative possibility—that there is linkage between these two traits with sufficient crossing-over to account for the four individuals with only one trait—is unlikely. Two possibilities for the mode of inheritance of primordial dwarfism are mentioned in the introduction. The indisputable production of a normal individual from mating of two of these dwarfs would exclude simple recessive inheritance and would favor some other mechanism, such as that

proposed by Hogben (1932). However, I have been able to find no definite report of a normal individual born of two primordial dwarfs. The presumably normal child of General Tom Thumb and his dwarfed wife died in infancy.

In the family reported here both parents of the affected sibship are presumably heterozygous for both mutant traits. The relatively small stature of the father in particular may be an expression of heterozygosity: this is suggested by the small stature of certain parents in many other pedigrees of primordial dwarfism.

The expected incidence of homozygous recessives among the offspring from two heterozygotes would be one in four. The observed incidence was, however, about twice as great. The likelihood of four out of nine affected by one trait is 11.7%. The likelihood of this ratio (4/9) occurring independently for each of two traits is $\left(\frac{11.7)^2}{100}\right)$ or 1.4%. The likelihood of any given individual being affected by both traits is $1/4 \times 1/4$ or 1/16. The likelihood in a family of nine of finding two individuals both affected by both traits is about 9%. The findings in this sibship are, therefore, somewhat unusual although the calculated probabilities are still high enough to make the genetic hypothesis of independent recessive inheritance of the two traits credible.

When a heterozygous individual marries a first cousin the chance of the first cousin's being heterozygous for the same trait is one in eight and of a given child's being affected, one in 32. For an "intermediate double first cousin" these values become 3 in 16 and 3 in 64.

When an individual is heterozygous for two independent traits the chance of both of them being carried by a first cousin is 1 in 64 and in an "intermediate double first cousin" 9 in 256.

The sibs of the recessive homozygotes in this family have a one in three chance of being free of either one of these genes considered independently and only a one in nine chance of carrying neither of the mutant genes. The chance of a member of sibship V 14–17 being affected with one or both of these pathologic traits cannot be estimated since the frequency of neither of the responsible genes in the population is known. A gene frequency of 1 in 200 for the general population would not be a startling incidence. Using this entirely credible figure, the probability of an individual in sibship V 14–17 being affected with one trait then becomes 1 in $300\left(2/3 \times \frac{1}{200}\right)$. In fact the probability may be appreciably greater since (see the pedigree) the parents of the sibship in question are distantly related.

Primordial dwarfism

The intelligence of primordial dwarfs, like that in achondroplasia, is normal. These Lilliputians are usually "music hall artists", vaudevillians, or some other modern version of the role their dwarfed forebears filled as court pages and retainers of the nobility and royalty. Some of the historically known dwarfs attained reputations in the arts. Aesop, author of the *Fables*, was reputedly a dwarf of about the size of those described above but his type of dwarfism is, of course, unknown.

Sexual infantilism occurs in some. Although fertility is considerably reduced, sterility is by no means the rule. The attainment of sexual maturity is usually delayed, however. On record are numerous instances of children born to parents, one or both of whom were primordial dwarfs. Naegele, famous German obstetrician, described (1839) the uneventful delivery of a primordial dwarf. Obstetrical complications are the exception.

The life-span of these individuals probably does not deviate significantly from the normal. Count Joseph Bornwlaski, who was 39 inches tall, died at 98 years of age.

Radiologically and autoptically, a delayed union of epiphyses, as well as their delayed appearance and even absence, is characteristic. The dentition shows the same tardy development. The deciduous teeth may not be lost until the third decade of life. Charles S. Stratton ("General Tom Thumb") had a double row of teeth, the deciduous teeth having persisted after the development of his permanent set. Third molars are frequently absent.

It is possible that ateleiotic dwarfism does not constitute a homogeneous group pathogenetically. Although some persons would include individuals with sexual infantilism in the same general group as the primordial dwarfs, in my own opinion the term should be reserved for cases with normal or near-normal sexual development, like those reported here. Even excluding cases of sexual infantilism there may be a fundamental heterogeneity of the group, since one pedigree of Schaefer and Strickroot (1940), with six affected persons, appears to demonstrate dominant inheritance through three generations.

The possible role of the pituitary in proportionate dwarfism is uncertain (Horstmann, 1950). The defect would need to be limited to a deficiency of growth hormone. Genetic dwarfism in certain strains of mice is due to an inherited defect of the eosinophilic cells of the anterior pituitary (Smith and MacDowell, 1930). In fact, the mechanism of ateliotic dwarfism in man is unknown. Needed are assays for growth hormone in the blood by some method such as that used by Segaloff.

Primordial dwarfism has been recognized in many species, such as horses (Rischbieth and Barrington, 1912), Jersey cattle (Mead, Gregory and Regan, 1942), and guinea pigs (Sollas, 1909). The King Charles spaniel and the bantam fowl are further examples. In most of these instances the trait follows a recessive pattern of heredity.

Some of the pygmies of the Congo are said to be achondroplastic, whereas the members of another tribe are normally proportioned. However, since they average about 54 inches in height, it seems improbable that the dwarfism in the Negro pedigree discussed in this paper represents a "throw-back" to pygmy ancestry.

Ectopia lentis

Although with the passage of years subluxation of the lenses may become more evident (possibly the accumulation of the minor traumata is a factor), the ocular abnormality, if present, almost certainly would have been detectable, at least by the slit lamp examination, in individuals IV 14 and IV 18.

In addition to the three main genetic categories of ectopia lentis listed in the introduction, a fourth represented by its association with the Ehlers-Danlos syndrome, another heritable disorder of connective tissue, has been described (Thomas *et al.*,

1952) in at least one patient. In this instance, however, further similar observations must be awaited before the association can be considered more than coincidence.

SUMMARY

Ateliotic dwarfism and ectopia lentis are described in each case in four members of a sibship produced by a consanguineous mating. The pedigree is consistent with a recessive and independent mode of inheritance of both traits. The relatively small stature of the father and of some members of the sibship (i.e., individual IV 19) may be an expression of heterozygosity for the dwarf trait.

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The Chances of Excluding Paternity by the MNS Blood Group System[1]

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WIENER (1952) HAS CALCULATED the chances of exonerating a man of a false charge of paternity by means of the MNS blood groups. Wiener's formulas apply, however, to the case in which the bloods are tested with four sera, anti-M, anti-N, anti-S and anti-s. In actual practice the anti-s serum is usually not available, and the tests are done with three sera only. It would be desirable to be able to calculate the chances of exclusion in this case.

Formulas similar to those given by Wiener can be derived for the probabilities when the tests are done with three sera. If we represent, as does Wiener, the frequency of the genes MS, NS, Ms, and Ns by the letters a, b, c, and d respectively, and let $m = a + c$, $n = b + d$, $p = a + b$, and $q = c + d$, the formulas are:

$$
\begin{aligned}
P_{MS} &= n(1 - mn) \\
P_{M} &= n(1 - mn) + aq^2 + bcd \\
P_{MNS} &= 0 \\
P_{MN} &= pq^2 \\
P_{NS} &= m(1 - mn) \\
P_{N} &= m(1 - mn) + bq^2 + acd \\
P_{M,N,S} &= mn(1 - mn) + (ac^2 + bd^2)q^2 + 2cdpq^2 + cd(ad^2 + bc^2)
\end{aligned}
$$

where P_{MS} represents the probability of excluding the paternity of a man of group MS, P_{M} the probability for a man of group M, etc., and $P_{M,N,S}$ represents the chance of excluding paternity of an innocent man whose MNS blood group is not known.

For the English population used by Wiener to illustrate his calculations, which is sufficiently similar for our purposes to American populations, (data in Race and Sanger) we have $a = 0.2472$, $b = 0.0802$, $c = 0.2831$, $d = 0.3895$, and the probabilities are as follows:

Group of Man	Probabilities of Excluding Paternity Using Three Sera
MS	0.3527
M	0.4734
MNS	0
MN	0.1481
NS	0.3982
N	0.4618
Unknown	0.2390

[1] The research reported in this paper was made possible in part by the use of equipment purchased by Boston University under contract No. Nonr-492 (01) with the Navy Department (Office of Naval Research).

For this population Wiener found that the chance of excluding paternity by the MNS system, when the group of the man is unknown, using four sera, is 0.315 (actually 0.3158). The chances using anti-M and anti-N alone are 0.1870, so it is apparent that the addition of the anti-S serum adds about 40% as much to the chances as does the addition of both anti-S and anti-s.

The value of the probability for the English population given by Race and Sanger (1954), and used by me in a previous paper (1954) is 0.2741, which is seen to be somewhat too high.

It was pointed out by Wiener (1952) that if the calculations for the MNS system, for the case in which four sera are used, are made using the simple but erroneous assumption that the MN and Ss systems are independent, the result is only a few per cent in error. In a previous paper (Boyd 1954) this method was described and used to estimate the probability for a population, and it was stated that the result was doubtless too high. It is remarkable how small the error actually is. For the English population referred to above the calculation making use of the erroneous assumption of independence gives 0.2415 instead of the exact 0.2390, an error of only 0.0025. For certain sets of gene frequencies the results of the two methods agree, and it can easily be shown that if the gene frequencies are connected by the relation $a/c = b/d$, the two methods will give identical results in the case of tests done with three sera. For certain other gene frequencies the approximate method may give a slightly smaller result than the exact method, as is found for the data from Lahore (in Boyd and Boyd 1954), where the approximate method gives 0.2428 and the exact method 0.2430. For the similar but hypothetical gene frequencies $a = 0.3$, $b = 0.1$, $c = 0.4$, $d = 0.2$, the difference in this direction is somewhat larger, *viz.* 0.0011. In most cases, however, the results of the approximate method are larger, but seldom more than 0.0200 greater than those of the exact method.

It is evident that the calculations done by the simpler method are close enough to the truth for all practical purposes, and under exceptional circumstances may be exact. It is not easy to see how this could have been ascertained, however, without first deriving the correct formulas.

SUMMARY

Formulas are given for calculating the chances of excluding paternity of a falsely accused man by means of the MNS blood group system, when the tests are done with anti-M, anti-N and anti-S sera. It is pointed out that the approximate calculations based on the erroneous assumption that the MN and Ss systems are independent produces but a very small error in the estimates in this case.

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Sex-Linked Hereditary Deafness

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ALTHOUGH IT HAS been established that deafness may be hereditary, most efforts to determine the probability of hereditary deafness for any one child in a family usually have been unsuccessful. This has been primarily due to the difficulties in accurately diagnosing individual cases of profound nerve deafness and in establishing the categories to which the parents belong. The literature does not disclose any previously reported cases of true sex-linked congenital nerve deafness. In the excellent and comprehensive report on pedigrees from the Clarke School[1] there is no evidence of any sex-linked hereditary deafness. The rarity of such a condition has been confirmed by Hopkins (2) and Fowler (3) who have had extensive opportunities to encounter such cases.

A family tree, figure I is, therefore, presented, which seems to indicate some interesting genetic aspects of sex-linked hereditary deafness.

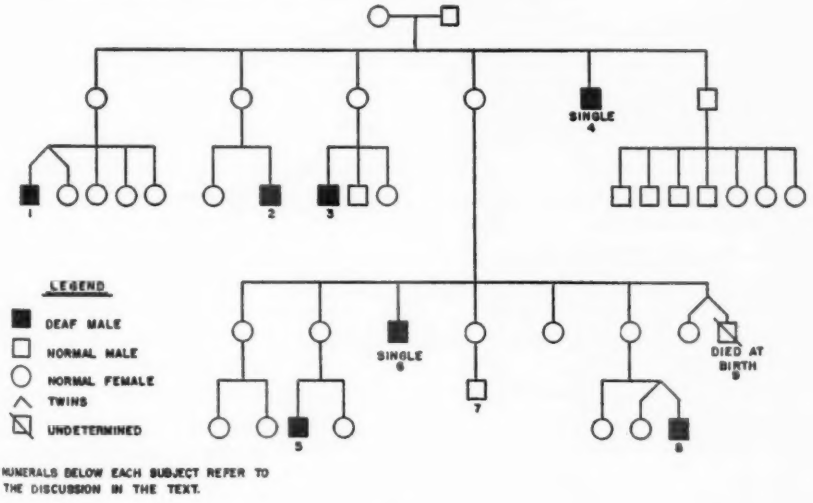

FIG. I

Audiograms were available for this family on all of the living persons with profound congenital nerve deafness. Not all the members of this family have been given hearing tests, but those reported as not deaf have normal speech and clinically normal hearing as established from information supplied by close relatives.

Received November 10, 1954.

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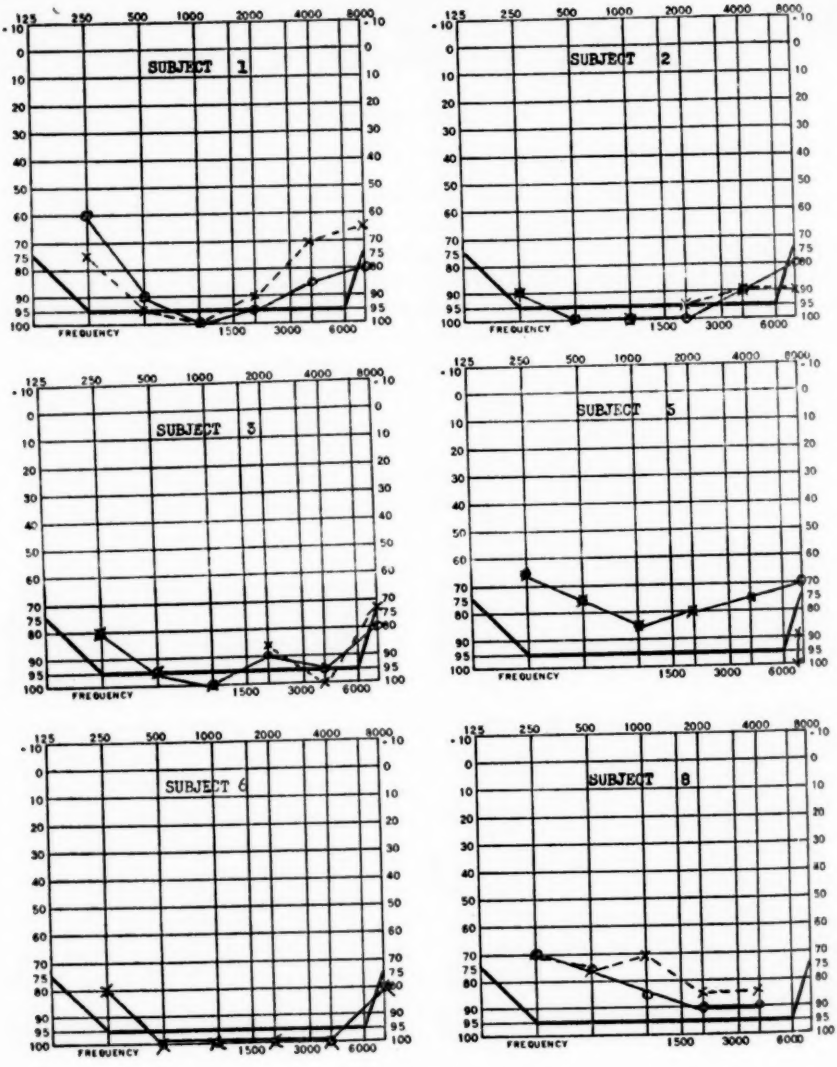

FIG. II

Figure II shows the audiograms obtained on six of the seven deaf individuals in the family.

An audiogram is a graphic record of an individual's hearing loss. Normal hearing for each of the tested frequencies lies between 0 and 10 decibels below the average normal hearing line or "0" decibel. The higher the decibel loss in each frequency

(further from the zero line) the greater the hearing loss. An individual with a nerve deafness over 75 decibels has very little useful residual hearing even with a hearing aid. The severity of the hearing losses shown in Figure II is consequently very great.

Subject 1 (In Figures I and II) Is now 53 years of age and has little intelligible speech. He was the first born and only male child in his family and has a twin sister.

Subject 2 Is a 44 year old deaf mute. He is the first born and only male.

Subject 3 Is a 37 year old first born male who received his education at a school for the deaf.

Subject 4 Disappeared over forty years ago at the age of 7. At that time he was known to be deaf and unable to speak.

Subject 5 Is a 16 year old now attending a school for the deaf. He is the first born son and has a sister, whose hearing is normal. He uses a hearing aid with fair results and has fairly intelligible speech. The usefulness of early amplification and adequate training is evident in this subject.

Subject 6 Is a 37 year old bachelor classified as a deaf mute with little intelligible speech.

Subject 7 Is a five year old first born male child with normal hearing and speech. He was conceived after a five year period of apparent sterility requiring a D & C. The mother denies any miscarriages prior to subject's birth or abortions.

Subject 8 Is a three year old first born male and has a twin sister. He has no speech and has profound nerve deafness. Both his twin sister and older sister have normal hearing and speech development. The hearing threshold on this youngster was obtained with repeatedly consistent psycho-galvanic skin resistance tests.

Subject 9 Was a male twin who died at birth.

There are three sets of twins in the pedigree. In each set there is a male and female, and the male was the first born son and demonstrated deaf mutism in two of the three sets.

It is apparent from this pedigree deafness is manifest only in the male child and is transmitted through the maternal side. There are seven first born males with profound congenital nerve deafness, a condition not present in subsequently born males or found in any females. There are insufficient subsequent males to establish statistically that deafness is restricted to the first born males. Deafness does not exist in the children of the normal male member of the pedigree.

This family seems to demonstrate sex-linked nerve deafness of the recessive heredity type, similar to that seen in hemophilia. There is no history of any consanguinous marriages in the family. There is an excellent probability in this family that approximately one-half the males born after an affected male may be expected to be deaf. Although it is possible that there may be other effects of the gene in the heterozygous condition, none was apparent in this family. Allied conditions; such as Rh incompatability and syphilis were ruled out as etiologic possibilities.

The family pedigree exhibited demonstrates clearly that profound congenital nerve deafness can be hereditary and sex-linked. Early recognition and adequate educational measures are essential in such instances.

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A Study of Variations in the Frequency of Twin Births by Race and Socio-Economic Status

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ONE OF THE PROBLEMS concerning twinning is the existence of variation in the frequency of twin births in various ethnic and racial groups. Most recently, Guttmacher (1953) has reviewed the literature on this subject. Of the 12 countries from which data have been reported, Norway has the highest frequency of twin births, 14.5 per 1,000 total births, while Japan has the lowest, 6.5 per 1,000 total births. In the United States, differences in the frequency of twinning between the white and non-white segments of the population have been recorded by Hamlett (1935) and Strandskov and Edelen (1946). An analysis of the annual reports of the United States National Office of Vital Statistics has shown that the frequency of twin births in the white population was 11.3 per 1,000 total births as compared to 14.3 per 1,000 total births among the non-white population. Of additional interest is the observation that the frequency of monozygotic twins did not differ very much in the two population groups, being 3.9 per 1,000 among whites and 4.1 per 1,000 among non-whites, whereas the frequency of dizygotic twin births differed markedly, being 7.4 per 1,000 among whites as compared to 10.1 per 1,000 among non-whites. In general, it has been found that the differences noted between ethnic groups in the twinning tendency principally represent differences in dizygotic twin births with very little difference in monozygotic twin births (Komai and Fukuoka, 1936).

Since the frequency of dizygotic twins varies with maternal age and birth order, while that of monozygotic twins does not (Yerushalmy and Sheerar, 1940), the question must be raised as to whether the differences observed between white and non-white groups merely reflect, in whole or in part, possible differences in the maternal age and birth order patterns in these groups. It is also possible that the differences between the white and non-white populations in the United States may be a manifestation of the different socio-economic circumstances in which these two groups live. This report presents an analysis of data obtained from birth records in Baltimore in an attempt to study variations in the frequency of monozygotic and dizygotic twin births among white and non-white segments of the population and further to compare whites and non-whites after maternal age, birth order and socio-economic status have been taken into consideration. In addition, it will be possible to note whether or not there exist variations in the frequency of the two types of twin births by socio-economic status since the existence of such variations must be

Received January 6, 1955.

taken into consideration in evaluating the use of twin studies for testing genetic hypotheses.

METHOD OF STUDY

In Baltimore, during the period 1941 to 1948, there were recorded in the Baltimore City Health Department a total of 154,550 births, including live- and stillbirths. Of these, 41,013, or 26.5 per cent, were non-white births consisting almost entirely of Negro births. On the birth certificates the age of mother, birth order and race are routinely recorded, thus making it possible to adjust for these variables in making comparisons between different population groups. In addition, Baltimore is one of the metropolitan cities on which the Bureau of Census publishes information concerning characteristics of the census tracts. The census tract comprises a neighborhood of between 3,000 and 6,000 individuals who are relatively homogeneous with regard to certain population characteristics. For each census tract there are available certain socio-economic indices, such as median monthly rental, occupational data, home ownership, etc., by means of which the population may be classified into socio-economic groups. It is thus possible, by using information available on birth certificates in Baltimore, to study the variations in the frequency of twinning by race and socio-economic groups.

A limitation to this method of socio-economic classification should be briefly considered. Table 1 contains the frequency of first births and of births occurring to mothers under 25 years of age by race and a division of the population into fifths according to socio-economic level. From this table we can see that 73% of the non-white births are classified in the lower two socio-economic fifths. A majority of the remaining 27% non-white births, although classified in the higher categories, probably live in socio-economic circumstances that more closely approximate the lower two socio-economic fifths. This might occur when several blocks of non-white families are located in a census tract that is predominantly white and are, therefore, classified in one of the higher socio-economic groups although the socio-economic status of the non-white families may be more equivalent to that of the lowest socio-economic fifth in the general population. This results from a method of classifica-

TABLE 1.—FREQUENCY OF FIRST BIRTHS AND OF BIRTHS OCCURRING TO MOTHERS UNDER 25 YEARS OF AGE, BY RACE AND SOCIO-ECONOMIC FIFTHS

Socio-Economic Fifths	White Births			Non-White Births		
	No. of total births	Per cent first births	Per cent occurring to mothers under 25 years of age	No. of total births	Per cent first births	Per cent occurring to mothers under 25 years of age
1 (lowest)	21,091	37.1	50.8	14,683	29.1	61.7
2	17,376	40.2	49.5	15,151	33.8	65.9
3	23,840	44.5	48.1	8,056	31.8	57.4
4	23,650	50.1	42.2	2,635	38.8	56.4
5 (highest)	27,580	50.5	33.3	488	—	—
Total.....	113,537	45.1	44.0	41,013	31.8	60.2

tion based on average characteristics of individuals living in an area rather than on actual individual characteristics. However, an analysis of data based on census tract classification does provide suggestive leads that indicate the problems to be studied by more refined methods of investigation.

Because of the influence of maternal age and birth order on the frequency of dizygotes, it was necessary to see if the groups being compared were different with regard to these characteristics. Space does not allow a detailed presentation of these distributions, but in Table 1 there are presented the percentage of total births that had occurred to those mothers who were under 25 years of age and the percentage of first births by race and socio-economic fifths. From this table, we may note that among whites the percentage of first births increases from 37.1 per cent in the lowest socio-economic group to 50 per cent in the highest socio-economic group. On the other hand, the percentage of total births occurring among those mothers who were under 25 years of age decreases from 50.8 per cent in the lowest group to 33.3 per cent in the highest group. Among non-whites there is a suggestion of a similar pattern but it is less marked and more irregular.

In making comparisons between races and socio-economic groups, differences in maternal age and birth order distributions can be taken into account by a method of adjustment similar to that used for age adjustment in routine vital statistics practice. For each race the like-sexed and unlike-sexed twin frequencies were calculated for each birth order and maternal age group. The birth order groups used were 1, 2, 3, and 4 and over, and the maternal age groups in years of age were, under 20, 20–24, 24–29, 30–34, and 35 and over. The maternal age and birth order specific frequencies for like-sexed and unlike-sexed twin births were multiplied by the total white births in each maternal age and birth order class and expected numbers were obtained. For each racial group, these expected numbers were totaled and, when these totals were divided by the total white births, a maternal age and birth order adjusted frequency was obtained. Thus the non-white rates were adjusted to the white maternal age-birth order distribution. These rates are, therefore, comparable with each other and will not reflect differences in maternal age and birth order. An estimate of the frequency of monozygotic and dizygotic twins was obtained by applying Weinberg's Differential Method to these expected numbers of like- and unlike-sexed twins in each socio-economic class and racial group. In using Weinberg's method, the sex ratio was taken to be 1, since it was thought that use of the actual sex ratio would affect the results only slightly.

In order to carry out statistical tests of significance of the differences between the groups being studied, a method of statistical analysis recently proposed by Cochrane has been used (Cochrane, 1954). The twin frequencies within each maternal age-birth order category are computed and compared and the results of the comparisons from all of the maternal age-birth order groups are pooled for a combined test of significance. Allowance is thereby made for the effect of maternal age and birth order on the twin frequencies. We shall illustrate the use of this method by an example in which we compare the frequency of like-sexed twins among whites and non-whites. The necessary data for one particular maternal age-birth order category (under 20, birth order 1) are presented in Table 2. For this category we

TABLE 2.—FREQUENCY OF LIKE-SEXED TWIN BIRTHS OF FIRST BIRTH ORDER AMONG MOTHERS UNDER 20 YEARS OF AGE BY RACE

Race	Total	Like-Sexed Twin Births	
		No.	Frequency
White Births	8971	37	.0041 ($=p_w$)
Non-White Births	7255	41	.0057 ($=p_{nw}$)
All Births	16226	78	.0048 ($=p_t$)

calculate the frequency of like-sexed twins among whites, non-whites and for the total (p_w, p_{nw} and p_t). We then compute the difference between non-whites and whites ($d = p_{nw} - p_w$) with the direction of the difference being indicated. Thus, in this example $d = +.0016$. A weighting factor for this category is calculated from the numbers of the two groups, as follows:

$$\text{Weighting factor} = W_1 = \frac{n_w\, n_{nw}}{n_w + n_{nw}} = \frac{8971 \times 7255}{8971 + 7255} = 4{,}011.1$$

These computations are carried out for each of the maternal age-birth order categories. A weighted mean difference ($\bar{d}$) is then computed by summing these for all the categories, thus,

$$\bar{d} = \frac{\Sigma W d}{\Sigma W}$$

This has a standard error

$$S.E. = \frac{\sqrt{\Sigma W p_t q_t}}{\Sigma W}$$

The probability level of the difference is determined by computing $\bar{d}$/S.E. and referring to the tables of the normal distribution.

In presenting the results of this study, the data are presented in terms of adjusted rates of like- and unlike-sexed twins and of monozygotic and dizygotic twins for visual comparisons, and the actual statistical analysis is carried out by summing the comparisons made in each maternal age-birth order category and applying the test devised by Cochrane.

RESULTS

During the period 1941 to 1948, there had occurred 1,142 white twin births representing a frequency of 10.1 per 1,000 total births as compared to 529 non-white twin births with a frequency of 12.9 per 1,000 total births. The frequency of white monozygotic twin births was 4.4* as compared with 4.3 among non-whites, which is in agreement with previous observations in that there is very little difference between races with regard to monozygotic twinning (Guttmacher, 1953; Hamlett,

* In this report all frequencies are expressed as per 1,000 total births.

TABLE 3.—MATERNAL-AGE AND BIRTH ORDER ADJUSTED FREQUENCIES FOR MONOZYGOTIC AND DIZYGOTIC TWIN BIRTHS BY RACE

Race	Expected No. of		Monozygotic Twin Births*		Dizygotic Twin Births*	
	Like-sexed twin births	Unlike-sexed twin births	Expected number	Adjusted frequency (per 1,000)	Expected number	Adjusted frequency (per 1,000)
White Births	821	321	500	4.4	642	5.7
Non-White Births	1035	449	556	4.9	898	7.9

* Estimated by Weinberg's Differential Method.

1935; Strandskov and Edelen, 1946). However, the estimated frequency of dizygotic twins among whites was 5.7 as compared to 8.6 among non-whites, a difference which is also in general agreement with previous observations. In order to take the difference in the maternal age and birth order distribution of the births into account, the maternal age birth order adjusted frequencies were computed for whites and non-whites. The monozygotic non-white adjusted frequency was 4.9 as compared to 4.4 for the white births and the non-white dizygotic adjusted frequency was 7.9 as compared to 5.7 for the white births (See Table 3). Thus, the process of adjustment increased the difference in frequency for monozygotes while decreasing the difference for the dizygotes.

Tests for statistical significance were carried out by comparing the frequencies of like-sexed and unlike-sexed twin births and the estimated frequencies of monozygotic and dizygotic twins among whites and non-whites. The results are presented in Table 4. From this we note that the frequencies of like-sexed and unlike-sexed twin births are higher among non-whites and these differences are significant at probability levels of .02 and .01, respectively. After the monozygotic and dizygotic twin births are estimated and their frequencies compared, the probability levels change. For monozygotic twins, the frequency is still higher among non-whites but it is not statistically significant ($P = .11$). On the other hand a comparison of dizygotic frequencies indicates an increased frequency among non-whites and this difference is significant at a probability level of .004. In evaluating these results it is necessary to keep in mind the fact that there is a certain error associated with using Weinberg's method. In addition, in carrying out these significance tests for mono-

TABLE 4.—MEAN DIFFERENCE OF FREQUENCIES OF VARIOUS TYPES OF TWIN BIRTHS BETWEEN WHITE AND NON-WHITE BIRTHS

Type of Twin Birth	Weighted Mean Difference Per 1,000 Births (Non-Whites Minus Whites)	Standard Error of Mean Difference	Ratio: $\frac{\text{Difference}}{\text{S. E.}}$	P
Like-Sexed	+1.3*	0.56	2.32	.02
Unlike-Sexed	+0.9	0.36	2.53	01
Monozygotic	+0.7	0.44	1.59	.11
Dizygotic	+1.5	0.52	2.88	.004

* A plus sign indicates that frequency is higher among Non-Whites.

and dizygotes on each maternal age-birth order category, it was necessary to eliminate several categories where the number of unlike-sexed exceeded the number of like-sexed twin births, in which situation it is not possible to estimate the number of monozygotic and dizygotic twin births. It is, therefore, necessary to consider all of the data when interpreting these results. For example, we are interested in like-sexed differences since they afford an indication with regard to monozygotic frequencies. If like-sexed differences are not significant at the usual probability levels whereas the estimated monozygotic differences are significant, we can only consider the evidence as being suggestive. On the other hand, if both of these differences are significant, our confidence in the existence of such a difference is thereby increased. Thus from the comparisons made, it is possible to state that these two races definitely differ with regard to the frequency of dizygotic twins but that the difference with regard to monozygotic twins might be considered as being suggestive.

We know that there exist socio-economic differences between these two races. It was, therefore, considered desirable to see if the twin frequencies varied by socio-economic status since if there was such a variation it should be taken into consideration in comparing the two races. The twin frequencies for each socio-economic group and race are presented in Tables 5 and 6. In Table 5 they are unadjusted for maternal age and birth order whereas in Table 6 they are adjusted.

The white monozygotic and dizygotic adjusted frequencies are presented in the upper half of Table 6. From this we note that the monozygotic frequency among

TABLE 5.—FREQUENCY OF MONOZYGOTIC AND DIZYGOTIC TWIN BIRTHS, BY RACE AND SOCIO-ECONOMIC FIFTHS

Socio-Economic Fifths	No. of total births	Number of		Monozygotic Twin Births*		Dizygotic Twin Births*	
		Like-sexed twin births	Unlike-sexed twin births	Number	Frequency (per 1,000)	Number	Frequency (per 1,000)
White Births							
1 (lowest)	21,091	131	76	55	2.6	152	7.2
2	17,376	124	50	74	4.2	100	5.8
3	23,840	190	65	125	5.2	130	5.5
4	23,650	163	58	105	4.4	116	4.9
5 (highest)	27,580	213	72	141	5.1	144	5.2
Total	113,537	821	321	500	4.4	642	5.7
Non-White Births							
1 (lowest)	14,683	134	61	73	5.0	122	8.3
2	15,151	139	71	68	4.5	142	9.4
3	8,056	58	33	25	3.1	66	8.2
4	2,635	14	9	5	—	18	—
5 (highest)	488	8	2	6	—	4	—
Total	41,013	353	176	177	4.3	352	8.6

* Estimated by Weinberg's Differential Method.

TABLE 6.—MATERNAL-AGE BIRTH ORDER ADJUSTED FREQUENCIES FOR MONOZYGOTIC AND DIZYGOTIC TWIN BIRTHS, BY RACE AND SOCIO-ECONOMIC FIFTHS

Socio-Economic Fifths	Expected No. of		Monozygotic Twin Births*		Dizygotic Twin Births*	
	Like-sexed twin births	Unlike-sexed twin births	Expected number	Adjusted frequency (per 1,000)	Expected number	Adjusted frequency (per 1,000)
White Births						
1 (lowest)	645	377	268	2.4	754	6.6
2	783	346	437	3.8	692	6.1
3	922	311	611	5.4	622	5.5
4	762	266	496	4.4	532	4.7
5 (highest)	885	333	552	4.9	666	5.9
All White Births	821	321	500	4.4	644	5.7
Non-White Births						
1 (lowest)	1270	341	929	8.2	682	6.0
2	950	657	293	2.6	1314	11.6
3	953	351	602	5.3	702	6.2
4	—	—	—	—	—	—
5 (highest)	—	—	—	—	—	—
All Non-White Births	1035	449	556	4.9	898	7.9

* Estimated by Weinberg's Differential Method.

whites is lowest in the lowest economic fifth, and there is a suggestive pattern of increase with increasing socio-economic status. It must be admitted that the pattern of increase in frequency with increasing socio-economic status is not marked. On the other hand, the adjusted dizygotic frequency is highest in the lowest economic fifth and the frequencies decrease with increasing economic fifths until the highest socio-economic fifth, when it suddenly rises. The variations observed with regard to the adjusted dizygotic frequencies are not as great as those found in the case of the white monozygotes.

In order to apply the statistical test of significance to these data, it was necessary to create a dichotomy within each maternal age-birth order category. This was accomplished by combining the upper 2 economic fifths into one group and the lower 2 economic fifths into another. Thus, the twin frequencies in the upper economic two-fifths are compared with those in the lower. The results of these comparisons are presented in the upper half of Table 7. From this we note that the frequency of like-sexed twins is higher in the upper economic group and the difference between upper and lower economic strata is significant at a probability level of .03. On the other hand the frequency of unlike-sexed twins is lower in the upper economic group but the difference is not statistically significant. A comparison of the estimates of monozygotic twin frequencies in these groups indicates that it is higher in the upper economic group at a probability level of .0014. The dizygotic twin frequency is lower

in the upper economic group and the difference is significant at a probability level of .02. It would appear from these comparisons that monozygotic twins are more frequent in the upper economic groups and that the significance level for this difference probably lies somewhere between .03 and .0014. On the other hand, the increased frequency of dizygotic twins in the lower economic groups can be considered only suggestive in line with the considerations previously discussed.

The non-white frequencies similarly adjusted are shown in the bottom half of Table 6. We note that the frequencies fluctuate markedly. The monozygotic frequency is highest in the lowest fifth with a rate of 8.2 and the lowest frequency is 2.6 in the second economic fifth, with no particular pattern of change with regard to socio-economic status. Frequencies for the fourth and fifth economic groups were not computed because of the small number of non-white births in these groups. The variations for dizygotes is as great as that found in the case of monozygotes. A major reason for these fluctuations is the smaller number of non-white births, which becomes particularly impressive when frequencies are computed for a particular maternal age- and birth order group. Among non-whites many of the maternal age-birth order specific frequencies are based upon less than a hundred births and so result in a great deal of sampling fluctuation.

In order to statistically analyze the variations in frequency between the socio-economic groups of the non-white population, it was necessary to combine the upper 3 economic-fifths into one group and to compare these with the lower 2 economic-fifths, since the number of births in the upper 2 economic-fifths were so small. The

TABLE 7.—MEAN DIFFERENCE OF FREQUENCIES OF VARIOUS TYPES OF TWIN BIRTHS BETWEEN UPPER AND LOWER ECONOMIC GROUPS BY RACE

Type of Twin Birth	Weighted Mean Difference Per 1,000 Births (Upper Fifths Minus Lower Fifths)	Standard Error of Mean Difference	Ratio: $\frac{\text{Difference}}{\text{S.E.}}$	*P*
		White Births[2]		
Like-Sexed	+1.3[1]	0.61	2.13	.03
Unlike-Sexed	−0.6	0.39	1.54	.12
Monozygotic	+1.6	0.50	3.2	.0014
Dizygotic	−1.3	0.55	2.36	.02
		Non-White Births[3]		
Like-Sexed	−1.6	1.00	1.60	.11
Unlike-Sexed	−0.2	0.73	0.26	.80
Monozygotic	−0.7	0.93	0.75	.45
Dizygotic	−2.0	1.13	1.77	.08

[1] A plus sign indicates that the frequency is higher in upper economic fifths, and a minus sign, that it is lower.

[2] Among whites, the comparison is between the upper economic two-fifths, and the lower two-fifths.

[3] Among non-whites the comparison is between the upper economic three-fifths and the lower two-fifths.

TABLE 8.—MEAN DIFFERENCE OF FREQUENCIES OF VARIOUS TYPES OF TWIN BIRTHS BETWEEN TOTAL NON-WHITE AND LOWER ECONOMIC GROUP WHITE BIRTHS

Type of Twin Birth	Weighted Mean Difference Per 1,000 Births (Total Non-White Minus Lower Whites)	Standard Error of Mean Difference	Ratio: Difference / S. E.	P
Like-Sexed	+2.0*	0.66	3.03	.0024
Unlike-Sexed	+0.9	0.46	1.96	.05
Monozygotic	+1.7	0.57	2.98	.0028
Dizygotic	+1.3	0.71	1.83	.07

* A plus sign indicates that the frequency is higher among Non-Whites than in the White lower economic two-fifths.

results of the analysis of the comparisons are presented in the lower half of Table 7. From this we note that none of the differences are sufficiently great as to ever reach a probability level of .05. The variations in the frequency of these various types of twin births among the economic groups of the non-white population are not statistically significant. From our earlier discussion of the method of economic classification used, this result is not unexpected.

In view of the differences in twin frequencies among white economic groups and since the socio-economic circumstances of the vast majority of the non-white population in Baltimore are more equivalent to the white lower economic groups, it appears necessary to take this into consideration in comparing the white and non-white twin frequencies. It appears reasonable to compare the non-white total frequencies with those in the white lower economic two-fifths. Thus we note that the total non-white adjusted monozygotic frequency is 4.9 as compared to the adjusted white frequencies of 2.8 and 3.4 in the two lower economic fifths and the total non-white adjusted dizygotic frequency is 7.9 as compared to the adjusted white frequencies of 6.6 and 6.1 for the lower two economic fifths.

The comparison of total non-white frequencies with white lower economic group frequencies was analyzed for statistical significance. The results are presented in Table 8. We note that the frequencies for all twin types are higher among non-whites than among whites. The difference of like-sexed and monozygotic twin frequencies is significant at probability levels of .0024 and .0028. On the other hand the unlike-sexed and dizygotic frequencies are different at probability levels of .05 and .07. Apparently the slight excess of unlike-sexed and dizygotic twins in the white lower economic groups as previously noted is sufficient to diminish the differences between the races to borderline statistical significance. From this comparison it appears that the monozygotic twin frequency is higher among non-whites than among whites when maternal age, birth order and socio-economic status are taken into consideration, whereas the dizygotic frequency is questionably higher.

DISCUSSION

The findings of this study suggest that the frequency of white monozygotic twin births is lower in the lower socio-economic segments of the population, while that of white dizygotic twin births is higher in the lower socio-economic segments with this

latter difference being of borderline statistical significance. These differences are present after adjusting for the variations in maternal age and birth order distributions between socio-economic segments. A comparison between whites and non-whites indicates that the frequency of monozygotes and dizygotes is higher among non-whites, after taking into account the effects of maternal age, birth order and socio-economic status; but the difference in dizygotic frequency is only of borderline statistical significance.

Before discussing possible explanations and implications of these findings, it is important to consider some limitations of the data. The comparisons of frequencies are made on the basis of adjustment for maternal age and birth order. It would have been more desirable to make direct comparisons of specific frequencies for each maternal age and birth order group between the various groups rather than in terms of an adjustment process. Unfortunately, the numbers were too small to permit this, particularly when broken down into different maternal age and birth order categories. The method of statistical analysis employed does not directly suffer from this limitation but, here again, it would have been preferable to compare frequencies directly without resorting to the pooling of the data for combined tests of significance.

Another possible limitation results from the use of Weinberg's method to estimate the frequency of monozygotic and dizygotic twin births. An aforementioned difficulty results from the use of census tracts as a means of economic classification. This is not the ideal method and cannot replace a classification by individual characteristics. But it is an inexpensive means of studying socio-economic differences which could indicate the need for more definitive studies. Despite these limitations, we think that the results are sufficiently suggestive to warrant the drawing of some inferences and the carrying out of more definitive studies to test these findings further.

The most surprising result was the variation in frequency of white monozygotic twin births among the different socio-economic groups. Even though statistical analysis indicated that this difference was highly significant (probability levels of less than .01), our interpretation is cautiously conservative in that we consider it as being not definitely established but highly suggestive requiring confirmation by other studies. The results are sufficiently suggestive to at least warrant an attempt at an explanation, however speculative it might be. One plausible explanation is that the difference may reflect higher abortion rates in the lower socio-economic groups. Since the frequency of abortions among monozygotic twins is probably higher than for single pregnancies, an increased abortion rate in lower economic groups would result in a lower frequency of monozygotic twin births. It would be necessary to obtain some reasonable estimates of abortion rates in these population groups to test this hypothesis. One difficulty with this hypothesis is that, since the frequency of abortions for dizygotic twin pregnancies is probably also higher than for single pregnancies we would expect that the frequency of dizygotic twin births by socio-economic groups would follow a pattern similar to that found for monozygotic twin births. However, since the frequency of abortions among dizygotic pregnancies is also probably less than for monozygotic pregnancies, the variation of frequency should not be as great as that noted for the monozygotes. Much depends upon the

relative differences between the frequency of abortions among monozygotic, dizygotic and single pregnancies. For example, if the frequency of abortion among monozygotes is much higher than among dizygotic and single pregnancies, while the abortion risk of dizygotic pregnancies is only slightly higher than of single pregnancies, we would not expect that the frequency of dizygotic twin births would be influenced to any great extent by varying abortion rates in different population groups. Unfortunately, the necessary data concerning abortion rates are not available. The only information that suggests that the differences in abortion rates may be in the above direction is that stillbirth frequencies are highest among monozygotes, less frequent among dizygotes and least among single births (Yerushalmy and Sheerar, II, 1940). It is obvious that the basic difficulty with any hypothesis is that we are actually interested in the frequency of twin pregnancies and not of twin births, since the latter are the end result of a period of intra-uterine existence during which many events may occur. At present, we have no good estimate of the frequency of occurrence of such events as abortions and consequently can only offer speculative hypotheses. Another explanation for the suggestive socio-economic differences which may be less probable is that the socio-economic segments may be composed of different ethnic groups which may have different twin frequencies. These possibilities must be kept in mind in any interpretation of the differences found.

Another interesting finding was that the frequency of monozygotic twin births was higher among non-whites than among whites, particularly when maternal age, birth order and socio-economic status were considered. It is difficult to postulate a biological explanation for this difference. An extension of the same kind of reasoning that was used above with regard to socio-economic differences in the case of monozygotes would imply that the racial differences might be a result of a lower abortion rate among non-whites. This does not seem very probable, but in the absence of actual estimates of such rates, the possibility must be kept in mind. If variation in environmental circumstances does not adequately account for the findings, it may be necessary to postulate the existence of genetic differences between these two races with regard to the monozygotic twinning tendency.

Of additional interest was the finding that the dizygotic twin frequency was higher among non-whites than among whites after the effects of maternal age and birth order had been taken into account. However, when the comparison between these two races was limited to comparable socio-economic groups, the difference in frequencies diminished from high to borderline statistical significance. This result is difficult to evaluate particularly since no marked differences were noted between the socio-economic groups of the white population. But it does raise the question as to whether the differences in dizygotic twin frequencies in various ethnic groups noted by other investigators are at least partially a result of environmental factors.

IMPLICATIONS WITH REGARD TO TWIN STUDIES

Admittedly, any biological explanations for the findings are purely speculative. However, these observations do have some practical implications with regard to the utilization of twin studies as a means of testing genetic hypotheses. It would appear

that a higher percentage of monozygotic twins would be in the upper socio-economic groups as compared to a group of dizygotic twins. If twin comparisons are being made to test a genetic hypothesis of a trait that is correlated either positively or negatively with socio-economic status, the comparisons in concordance frequencies between monozygotes and dizygotes may either overestimate or underestimate to some extent the true difference in concordance frequencies. If, in addition to these socio-economic differences, one considers the findings of other investigators, indicating that the monozygotes and dizygotes differ with regard to stillbirths rates, family size, and influence of maternal age, etc. the question must be raised as to their comparability. This becomes particularly important when these attributes are independently associated with the trait being studied, since it imposes certain restrictions on the inferences that can be drawn from twin studies. Some of these problems have been recently reviewed by Price, (1950).

An indication of some of these difficulties can be illustrated by the following simple mathematical model:

Let p_m = proportion of affected monozygotic twins among all monozygotic twins in the population, and

q_m = proportion of unaffected monozygotic twins among all monozygotic twins in the population, and

$p_m + q_m = 1$.

Likewise for dizygotic twins,

p_d = proportion of affected dizygotic twins among all dizygotic twins in the population, and

q_d = proportion of unaffected dizygotic twins among all dizygotic twins in the population, and

$p_d + q_d = 1$.

Then, p_m^2 = frequency of monozygotic pairs with two affected members, $2p_mq_m$ = frequency of monozygotic pairs with one affected member, and q_m^2 = frequency of monozygotic pairs with no affected members. This binomial distribution would hold true in a similar fashion for dizygotic twin pairs.

In using twins for studying a genetic hypothesis, the investigator selects an index twin with the condition being studied and then determines whether or not the other member of the twin pair is affected. He then subdivides his twin pairs according to whether or not they are monozygous or dizygous and for each of these types, he determines the percentage concordance, that is, the percentage of twin pairs in which both members are affected. The percentage concordance for monozygotes is compared with that for dizygotes and if significantly greater it is interpreted as being due to the influence of inheritance. Utilizing the terms of the binomial distribution, as derived above, it can be demonstrated that differences in concordance percentages may reflect differences between p_m and p_d.

In binomial terms, the percentage concordance for monozygotes can be expressed as follows:

$$\frac{p_m^2}{2p_mq_m + p_m^2} = \frac{p_m^2}{p_m(2q_m + p_m)} = \frac{p_m}{2q_m + p_m}$$

Similarly for dizygotes the percentage concordance can be expressed as,

$$\frac{p_d}{2q_d + p_d}$$

A comparison of these two ratios indicates that if p_m is greater than p_d, the percentage concordance for monozygotic twin pairs will be greater than the concordance for dizygotes. Thus a higher concordance percentage for monozygotes does not necessarily indicate genetic differences, since it may reflect differences between p_m and p_d. An example of such a situation was considered in a recent report of a study of epilepsy (Lilienfeld, and Pasamanick, 1954). In this investigation maternal and fetal factors such as certain complications of pregnancy and prematurity were found to be associated with epilepsy. It is thus possible that the greater prevalence of prematurity and perhaps other pregnancy factors among monozygotic than among dizygotic twin births may account, in whole or in part, for the greater concordance of epilepsy found in monozygotic twins.

One way of dealing with this problem is to determine the prevalence of a particular trait among monozygotic twins and among dizygotic twins rather than to start out with an index twin who is affected. Then one could determine if the concordance percentage is greater than is to be expected on a chance basis. Comparisons can then be made between monozygotic and dizygotic twins. If this is not done, one cannot be certain that the greater concordance among monozygotic twins is not a manifestation, at least in part, of an increased prevalence of the trait resulting from the characteristics of monozygosity.

SUMMARY

A study of the frequencies of mono- and dizygotic twin births born in Baltimore during 1941 to 1948 indicated that there is an increased frequency of both mono- and dizygotic twin births in the non-white population as compared to the white population, after adjustment for the influence of maternal age, birth order and economic status was made. An additional finding that can be considered as suggestive at present is that monozygotic twin births are less frequent in the lower socio-economic segments of the population. This finding requires confirmation by additional studies. Some possible explanations for these differences and their implications with regard to utilizing twin studies for testing genetic hypotheses are briefly discussed.

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BOOK REVIEWS

Evolution of the Vertebrates

By Edwin H. Colbert (The American Museum of Natural History and Columbia University), New York: John Wiley and Sons, Inc., 1955, Pp. xiii + 479, $8.95

MAN is able to study his history because of the intellect he has evolved. This is the theme that Dr. Colbert expresses and ably uses in bringing the reader through time from *Amphioxus* to *Homo*. Both of these forms are living today, but the millions of species produced through the epochs are mostly extinct, only a very few remaining to give man a record with which to speculate and deduce the trends of evolution.

The author does not restrict his treatment to the fossil record, but considers living forms in explaining the radiations of various vertebrate groups. Thus, the reader not only gets an understanding of the place of extinct forms, but gains an insight, clearly explained, into the relationship of extant vertebrates.

The author's lucid style makes the reader proceed without labor. The illustrations of phylogenies and skeletal structure are of top quality, easily understood, and greatly enhance the value of this book. The restoration drawings make such forms as *Eryops* and *Toxodon* come alive.

The anatomical considerations make this book valuable as a reference for the comparative anatomy of extant and extinct forms alike.

Information on particular groups, especially mammals, is discussed separately and then woven into the pattern of evolution through time. Man is not treated as the acme of evolution, but is placed in his proper place as the bud on a twig of the primate branch which may have at its base a form closely related to the primate-like insectivore, the modern Oriental tree shrew, *Taupaia*.

Dr. Colbert does not attempt to delve deeply into the realm of the anthropologist, but does mention the physical changes which have occurred. Within the Pleistocene, he believes that the evolutionary development of man was not of great magnitude, but was a matter of perfection of details that set man apart from other primates. He sets forth the thesis that there are four factors which were of prime importance in human development from an ape-like primate; the growth and elaboration of the brain, the perfection of the erect posture, the slowing down of post-natal development, and the growth of human populations. The latter, he relates to its importance in the human social structure and culture.

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Vaterschaftsbegutachtung

By H. Schade (Professor an der Universitat Munster, Westfalen). Printed by Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, 1954, Pp. 250 D.M. 28

DURING World War II many families were separated. After the war, families were reunited, but due to the passage of many years not infrequently uncertainties as to parentage arose. This situation in Germany was the inspiration for this book. In cases of disputed paternity and maternity, blood tests have become routine procedure in courts of New York City, and in other parts of the United States, as well as in various countries throughout the world. By combined use of the A-B-O, M-N, and Rh-Hr systems, a Caucasoid man falsely accused of paternity can be exonerated in about 55 percent of the cases. The author, Prof. Schade, is dissatisfied with blood tests because they do not yield decisive results in every case, and because they can be used only to exclude and not to prove parentage. This book represents an attempt to find other methods whereby an opinion can be offered regarding the parentage of a child in *every* case, based on external measurements of the body.

The book starts with a general discussion of the physical resemblance among members of the same family, and especially between uniovular twins. However, on page 46 there is a picture of a pair of uniovular twins, who look quite different because of an injury to the spinal column sustained by one of them. The author suggests a "method" to be used when offering an opinion regarding parentage in the court, using a "probability scale" ranging from zero to 100 percent. There are long detailed sections dealing with the shape of the face, scalp hair and eyebrows, skin, eyes and eyelids, nose, mouth and lips, ears, fingerprints, etc., while only nine pages are devoted to the entire field of blood grouping. Except in the case of the blood groups, no evidence is presented that the characteristics exhibit clear cut Mendelian inheritance. It is one thing to show that uniovular twins strongly resemble one another physically, and quite another to work out definite rules of heredity that can be applied for the determination of parentage.

At the end of the book is given a 10 page closely printed outline, recommending detailed measurements to be made and indices to be calculated for each individual examined. For example, more than a page is devoted to measurements and indices of the nose alone. At the end of the book is also given a list of physicians who, according to the author, are qualified to make these complex measurements for German courts and offer an opinion as to the parentage of a child.

It would not be difficult to write a devastating criticism of this book. Suffice it to point out that this is merely a revival of methods of physical anthropology which have proved unproductive in the past. In fact, it was not until blood grouping tests were introduced into physical anthropology, that it was possible to bring some order out of the confusion. The mechanism of inheritance of normal physical characteristics in man is, with few exceptions, unknown or poorly understood; moreover, the measurements of the body are strongly affected by age, nutrition, and other environmental factors, as in the case of the uniovular twins mentioned above. If any investigator were to make all the laborious measurements recommended in Prof. Schade's book, he would still remain entirely up in the air how to interpret the hundreds of measurements he had compiled regarding each individual. In fact, this is one time when the whole appears to be less than any of its parts. Even laymen realize that members of the same family frequently appear quite different, while there are instances where two unrelated individuals resemble one another very strongly. Therefore, in courts of this country, resemblance as a method of determining parentage was discarded long ago as too subjective and unreliable. This applies especially when comparing two individuals of different ages.

The book contains no scientific data in the way of family studies, to justify the use of physical measurements as a method of determining parentage. In fact, no rules of heredity have been worked out comparable to those available for the blood groups. The reviewer would like to see Prof. H. Schade subjected to a blind test, in which Prof. Schade would be required to examine and measure parents and children whose relationships are known but kept secret from him, and then let him decide which children belong to which parents. The value of the "blind test" in scientific investigations has become better recognized in recent years, as shown by the large scale experiments by the blind method in 1954 with polio vaccine. Prof. Schade's book will not be taken seriously by geneticists until he publishes the results of such a blind test or other objective evidence showing how physical body measurements can be used to solve problems of parentage.

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The Biochemistry of Semen

By T. Mann (University of Cambridge), London: Methuen & Co. Ltd.; New York: John Wiley & Sons, 1954. Pp. xiv + 240, $2.90.

This is a thorough and concise review and analysis of the work on the chemical physiology of semen, both mammalian and non-mammalian, beginning with the investigations of Leeuwenhoek in 1677 and including research as recent as 1953 of which the author himself has been a major contributor.

There are nine chapters in the book; the first three dealing with, (1) a general physiological description of the two components of semen (spermatozoa and seminal plasma), (2) the chemical

and physical properties of whole ejaculated semen, and (3), with the influence of internal and external environmental factors on the composition of semen. The last six chapters deal with the chemical composition and metabolism of various substances in the semen on a detailed basis. Chapter four is devoted to the proteins and enzymes in spermatozoa, while chapter five deals with the same substances in seminal plasma. Chapter six is concerned with the role of lipids in semen; chapter seven with the important subject of fructose and its metabolism; chapter eight considers the role of various amines and the last chapter discusses the significance of citric acid and inositol in semen.

The book will be of primary interest to the investigator and to those contemplating work in this field. The extensive bibliography, the many techniques that are detailed and evaluated, and the discussions of the numerous unanswered questions in the field would seem of utmost value. However, the physician will find items of interest here also, particularly the critical analyses of the various fertility tests based on semen composition and also the application of different tests of semen composition to forensic medicine. The human geneticist will do well to keep his eye on this rapidly expanding field; for if only a small portion of the inter-specific variation in semen composition reported in this book is reflected on an intra-specific level, then this would be a most promising field for genetic investigation.

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LETTERS TO THE EDITOR

This section is restricted to letters containing brief and pertinent comments on articles that have appeared in the Journal. The editor reserves the right to accept or reject any letter for publication.

March 30, 1955

To the Editor of the American Journal of Human Genetics

Dear Sir:

The article by Dr. C. C. Li in the December issue of this Journal presents, as is usual in his work, features of considerable interest. Since his method, despite its appeal on grounds of neatness and elegance, might be very difficult to apply to less simple situations, I think it worthwhile to bring to the attention of readers who may not be familiar with Wright's path coefficients their great utility for solving even quite complicated problems of the same general nature.

In the problem posed by Li, namely the derivation of an expression for the genetic correlation between parental means and the means of offspring in a randomly mated Mendelian population in equilibrium, assuming additive effects of genes, Wright's method may be applied directly considering merely that the variance of successive generations remains constant and that the means of large families approach the means of the corresponding parents. The variance of any generation is the sum of two components, the between family component which equals the variance of parental means (and which with random mating must be half the total variance), and the within family variance due to segregation of genes (which must constitute the remaining half of the variance). The variance of means of finite families containing s offspring is then $\sigma^2(1/2 + 1/2s)$, of which the first part, the between family component, may be attributed to parental means, while the second part is due to segregation. The path coefficient from parental means to family means is the proportion of the standard deviation that would remain if only that variation attributable to family means were operative (i.e., if all other causes of variation, namely that due to Mendelian segregation, were eliminated). This proportion is clearly $\sqrt{(\sigma^2/2)/(\sigma^2/2 + \sigma^2/2s)}$ or $\sqrt{s/(1+s)}$. Since there is no other correlated cause to be considered, this expression is also the correlation, in agreement with Li's results. The same expression was presented without derivation by Jay Lush in his book, "Animal Breeding Plans," 1937, Collegiate Press Inc., Ames, Iowa, on p. 134.

It must be admitted that the above is a "short cut" application of the path coefficient method; in fact, this particular problem can be solved as readily by direct use of the product moment formula, namely covariance divided by geometric mean of variances. Since the expected value of either one offspring or the mean of any number of offspring is equal to the parental mean, the covariance of parental and offspring means is simply the variance of parental means, or $\sigma^2/2$. Dividing this by the geometric mean of the variances, which are $\sigma^2/2$ and $(\sigma^2/2 + \sigma^2/2s)$, yields the same expression given above. The case of one parent and several offspring, also considered by Li, is as readily solved, taking the expected value of mean of offspring, when expressed as a deviation from the population mean, as one half the deviation of the parent considered.

In many genetic problems of this sort, where the groups of individuals whose correlation is to be calculated may be related through common ancestors, where mating may not be random, and where sources of nongenetic variance may exist, short cut methods are not obvious and it is worth while considering individual path coefficients from genotypes to phenotypes, from the genotypes of individual parents to those of individual offspring, from individual phenotypes to means, etc., and to obtain the desired correlations by summing all allowable paths. Once familiarity with the method is attained, these processes usually can be carried out step by step without great difficulty.

The general method of path coefficients is presented in Li's own book, "Introduction to Population Genetics," 1948 (distributed by O.S.C. Cooperative Association, Corvallis, Oregon), and by Sewall Wright in a number of publications. Among the latter may be mentioned a general derivation in the "Annals of Mathematical Statistics," V. 5, 1934, pp. 161–215, a concise and elegant but more difficult presentation in "Statistics and Mathematics in Biology" edited by Oscar Kempthorne, Iowa State College Press, Ames, Iowa, 1954, pp. 11–33, and a derivation with application to correlation

between relatives in Mendelian populations in "Genetics," V. 6, 1921, pp. 111–178. An interesting modification of the method in which regressions instead of correlations receive major emphasis, and one that sometimes leads to great simplicity, is presented by John W. Tukey in the volume mentioned above, "Statistics and Mathematics in Biology," pp. 35–66. There are numerous illustrations in the literature on animal breeding of the application of the method to obtain correlations between relatives and various combinations of relatives; among these might be mentioned one by L. N. Hazel, "Genetics", V. 28, 1943, pp. 476–490, and one by I. M. Lerner and the present writer in "Genetics," V. 32, 1947, pp. 567–579.

Sincerely yours,

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Errata

The following correction should be made in the article by Herman M. Slatis, entitled "A Method of Estimating the Frequency of Abnormal Recessive Genes in Man", which appeared in the December 1954 issue of this Journal. On pages 413–416, for $3/4^s$ read $(3/4)^s$.

BIBLIOGRAPHY OF HUMAN GENETICS

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THIS SECTION is a continued list of references to recent and current articles and books which may be of interest to students of Human Genetics. An attempt has been made and is being made to make the list both up to date and complete, but to do so is a difficult task. Everyone who finds the list useful and considers a complete one desirable, can be of help by sending to the bibliographic editor, at the address given above, any recent reference which has been missed or references to papers in press. The bibliographic editor wishes to take this opportunity to thank the many people who have been and it is hoped, will continue to be of help in this way.

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